Appeal No. 2015-1720, -1721

United States Court of Appeals

for the

Federal Circuit

GENZYME THERAPEUTIC PRODUCTS LIMITED PARTNERSHIP,

Appellant,

– v. –

BIOMARIN PHARMACEUTICAL INC.,

Appellee.

APPEAL FROM THE UNITED STATES PATENT AND TRADEMARK OFFICE

BRIEF FOR APPELLANT

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CERTIFICATE OF INTEREST

Counsel for Appellant certify the following:

1. The full name of every party or amicus represented by me is:

Genzyme Therapeutic Products Limited Partnership.

2. The names of the real party in interest represented by me is:

Not applicable.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

Genzyme Corporation.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court are:

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Dated: August 17, 2015

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TABLE OF ABBREVIATIONS

'226 PATENT	U.S. PATENT NO. 7,655,226
'226 PETITION	PETITION FILED IN IPR2013-00537 CHALLENGING THE '226 PATENT
'410 PATENT	U.S. PATENT NO. 7,351,410
'410 PETITION	PETITION FILED IN IPR2013-00534 CHALLENGING THE '410 PATENT
AIA	AMERICA INVENTS ACT
APA	ADMINISTRATIVE PROCEDURE ACT
BIOMARIN	APPELLEE BIOMARIN PHARMACEUTICAL, INC. (IPR PETITIONER)
BOARD	PATENT TRIAL AND APPEAL BOARD
ERT	ENZYME REPLACEMENT THERAPY
FDA	U.S. FOOD AND DRUG ADMINISTRATION
GAA	ACID α-GLUCOSIDASE
GENZYME	APPELLANT GENZYME THERAPEUTIC PRODUCTS LIMITED PARTNERSHIP (IPR PATENT OWNER)
IPR	*INTER PARTES* REVIEW
LSD	LYSOSOMAL STORAGE DISEASE
POSA	PERSON OF ORDINARY SKILL IN THE ART
PTO	U.S. PATENT AND TRADEMARK OFFICE

TABLE OF CONTENTS

Page

TABLE OF CONTENTS
(continued)

Page

TABLE OF AUTHORITIES

Page(s)

CASES

TABLE OF AUTHORITIES
(continued)

Page(s)

TABLE OF AUTHORITIES
(continued)

Page(s)

TABLE OF AUTHORITIES
(continued)

Page(s)

STATUTES

LEGISLATIVE AND ADMINISTRATIVE MATERIAL

TABLE OF AUTHORITIES
(continued)

Page(s)

STATEMENT OF RELATED CASES

The consolidated appeals before this Court derive from two *inter partes* review ("IPR") proceedings before the Patent and Trademark Office's Patent Trial and Appeal Board ("Board"): IPR2013-00534, which concerned U.S. Patent No. 7,351,410 ("the '410 patent") (Appeal No. 2015-1720) and IPR2013-00537, which concerned U.S. Patent No. 7,655,226 ("the '226 patent") (Appeal No. 2015-1721). BioMarin Pharmaceutical, Inc. ("BioMarin") was the petitioner and Genzyme Therapeutic Products Limited Partnership ("Genzyme") the patent owner in both IPRs.

Counsel for appellant Genzyme certify that no other appeal in or from the IPRs referenced above was previously before this or any other appellate court. Counsel further certify that there is no other case known to counsel to be pending in any court that will directly affect or be directly affected by this Court's decision in the pending appeal.

JURISDICTIONAL STATEMENT

On February 23, 2015, the Board issued final written decisions under 35 U.S.C. § 318(a). A1; A29.[1] Genzyme timely noticed appeals of those decisions on April 20, 2015. A13426-29; A13465-68; *see* 35 U.S.C. §§ 142, 319. This

[1] The Board's final decisions, along with the patents at issue and excerpts of relevant statutory and regulatory provisions, are included in an addendum to this brief.

Court has jurisdiction under 28 U.S.C. § 1295(a)(4)(A) and 35 U.S.C. § 141(c).

STATEMENT OF ISSUES

1. Whether the Board violated the administrative notice requirements applicable to formal adjudications under the Administrative Procedure Act by issuing final written decisions invaliding the challenged patent claims on legal and factual bases substantially different from those set forth in its institution decisions, which were the only notices the Board provided to Genzyme.

2. Whether the Board erred in construing the challenged patent claims as capable of covering the reduction or arrest of the further buildup of glycogen in liver alone, when the patents claim to treat human Pompe disease and it is undisputed that a person of ordinary skill in the art would have understood as of the date of the invention that human Pompe disease could not be treated only by arresting or reducing glycogen buildup in liver, but rather required—at a minimum—that effect in skeletal muscle.

3. Whether the Board's conclusion that the challenged patent claims are invalid on obviousness grounds is fundamentally flawed when its reasonable-expectation-of-success finding did not rely on any objective evidence of the perspective of a hypothetical person of ordinary skill in the art as of the date of invention, and when BioMarin submitted no such evidence.

STATEMENT OF THE CASE AND FACTS

A. Treatment Of Pompe Disease

The patents at issue claim methods of treating humans who suffer from Pompe disease. Pompe disease (also called glycogen storage disease type II or GSD-II) is a serious **genetic muscle disease** associated with a deficiency or absence of the lysosomal enzyme acid α-glucosidase ("GAA"). A3376; A4476; A954. In healthy individuals, GAA functions in the compartment of cells known as the lysosome to break down glycogen and produce glucose. A4476; A954. Patients with Pompe disease have markedly reduced levels of GAA, or lack GAA altogether. These patients thus are unable to break down glycogen, leading to its harmful accumulation in the lysosomes of skeletal muscles, as well as in the cardiac tissues of affected infants. A4476; A955.

As explained in the patents at issue, there are two clinical forms of Pompe disease: (i) early onset infantile, and (ii) late onset juvenile and adult. Early onset infantile Pompe disease presents shortly after birth and is associated with muscular weakness and cardiac failure, while "[s]ymptoms in the late onset [Pompe disease] in adult and juvenile patients occur later in life, and only skeletal muscles are involved." A1629; A885. Untreated, infants with Pompe disease rarely survive past their first birthday; patients diagnosed later in life often experience debilitating and degenerative myopathy (i.e., abnormality or disease of muscle tissue) and

respiratory symptoms. A4476-77; A955; A3323-24.

Physicians struggled for decades to develop a treatment for this serious disease. Beginning as early as 1964, scientists attempted to restore GAA levels in patients with Pompe disease by administering preparations that included GAA from sources external to the patient—an approach generally referred to as "enzyme replacement therapy" ("ERT"). A4477-78. But these efforts were unsuccessful. A3322 (BioMarin's expert agrees that "[p]rior to 1998, . . . there had been decades of failed attempts to treat Pompe disease using ERT"); A1200-01; A4477-80.

B. The Patents At Issue

A revolutionary advance in the treatment of patients with Pompe disease occurred with Genzyme's discovery of a method of treatment with alglucosidase alfa, which was approved by the Food and Drug Administration ("FDA") in 2006 as Myozyme® for use in infants with Pompe disease. A3848; A5079. In 2010, Genzyme received FDA approval for Lumizyme® (alglucosidase alfa) for use in the treatment of juveniles and adults with Pompe disease. A5540.

These two products were the first, and remain the **only**, FDA-approved products for treatment of Pompe disease. As BioMarin's own expert acknowledged, treatment of patients with Genzyme's Myozyme® and Lumizyme® products has "made a difference between life and death for many patients affected by Pompe disease." A3325; *see also* A5190 ("The development and regulatory

approval of Myozyme (alglucosidase alfa) for the treatment of Pompe disease represents the first major scientific and clinical breakthrough in the treatment of a life-threatening human myopathy."). In recognition of its groundbreaking work, Genzyme received numerous industry awards, including the 2006 Prix Galien Gold Medal, considered the pharmaceutical industry's equivalent of the Nobel prize. A4544-46; A5573; A5578; A5582.

The claims at issue—claim 1 of the '410 patent (A876-900) and claims 1 and 3-6 of the '226 patent (A1620-42)—cover the treatment of patients with Pompe disease with Genzyme's Myozyme® and Lumizyme® products when those products are used as directed in their product inserts. A4540-41. In particular, the claims are directed to methods of treating human Pompe disease patients by reducing or arresting the accumulation of glycogen. To understand the scope of the claims, it is necessary to understand the state of scientific knowledge concerning the treatment of Pompe disease as of December 7, 1998, the priority date here.

Pompe disease was one of more than thirty lysosomal storage diseases ("LSDs")—that is, diseases characterized by a deficiency in at least one lysosomal enzyme—identified as of December 7, 1998. A4575-76; A977; A4856; A4860-65. As of that date, ERT had been successfully developed for only **one** of these LSDs: Gaucher disease. A4577-78; A3226. The seminal Gaucher disease clinical trial

was published in 1991, more than seven years before December 7, 1998. A907-13.

Gaucher disease and Pompe disease were known to be very different diseases. Specifically, they are caused by a deficiency in different enzymes (glucocerebrosidase vs. GAA), involve different accumulated substrates (glucocerebroside vs. glycogen), affect different target tissues (macrophages (a type of white blood cell) vs. skeletal muscle and heart), and have different targeting mechanisms (mannose vs. mannose-6-phosphate receptor mediated uptake). A3242; A3267-69; A3271-76; A1223-25; A917.

The development of ERT for the treatment of Pompe disease, a muscle disease, was associated with "challenges that are different and more difficult" than those for Gaucher disease, specifically the significant challenges associated with delivering enzyme to skeletal muscle in human patients. A3279 (BioMarin's expert conceded "challenges that are different and more difficult [for Pompe disease] include the bulk of the skeletal muscle, the blood flow to the skeletal muscle, and the . . . relative paucity, of mannose-6-phosphate receptors in skeletal muscle versus that seen in the liver."). It was also recognized that *in vivo*—i.e., in human patients with Pompe disease—there would be competition for any administered GAA, especially by the liver, which would serve as an obstacle to effective treatment by preventing GAA from reaching the affected skeletal muscle or heart tissue. A4484-85; A4588; A1265. Experts in the field acknowledged this

obstacle, explaining that "receptor-mediated [ERT] for [Pompe disease] **may be feasible**, **if** high uptake forms of [GAA] would gain sufficient access to **affected muscle tissue**." A1265. BioMarin's own expert witness, Dr. Pastores, agreed with this assessment, A3369-71, and further conceded that *in vivo* (i) there would be competition from various sources for injected GAA, and (ii) the greatest uptake of GAA would have been expected to occur in the liver. A3369-70; A3341-42.

Indeed, the common constraint in all the previously discussed failed attempts to treat human Pompe disease was the observation that GAA was taken up by organs including the liver, **but not** by the key target tissues (i.e., the heart and skeletal muscles). A4480-81; A1102; A1429-30; *see also* A977 (noting "infused enzyme was found to be captured predominantly by the liver and the spleen when administered to animals or to patients. Uptake of enzyme in other organs was hardly observed"). Thus, as of December 7, 1998, there was "no effective treatment for Pompe disease." A997; *see* A3323. And ERT in particular had proved ineffective because it "normalized [GAA] and glycogen levels **in liver, but not in muscle and heart of patients with Pompe disease**." A997 (emphasis added); *see* A3378-79.

Moreover, it was understood that "**[t]o achieve therapeutic success**, the administered enzyme **must target** the lysosomes of the affected organs, **muscle and heart**." A997 (emphasis added). Or, as BioMarin's own expert

acknowledged, "to effectively treat Pompe disease," it was understood that the treatment must "target heart and skeletal muscle." A3274. In other words, as of December 7, 1998 (and indeed for decades beforehand) it was understood that the goal of treatment of Pompe disease was to reduce or arrest the further accumulation of glycogen **in the relevant target tissues**.

That goal was achieved through the work disclosed in the specification of, and claimed in, the '410 and '226 patents. The data demonstrates for the first time the long-term reduction/arrest of accumulation of glycogen in diseased muscle, which is captured by independent claim 1 of each of the patents, i.e., a "method of treating a human patient with Pompe's disease, comprising intravenously administering . . . a therapeutically effective amount of human [GAA], ***whereby*** **the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested**." A899; A1642 (emphasis added).

The scope of these claims—and particularly the "whereby" clauses—is clear from the scientific context in which the patents were prosecuted. As just explained, anyone skilled in the art in 1998 would have understood that "treating a human patient with Pompe's disease" can only be done by reducing or arresting glycogen buildup **in the relevant target tissues**. As explained in more detail below, and as confirmed by the specification and prosecution history, that is exactly what these patents claim. *See infra* Part III.

C. Proceedings Below

1. Inter Partes *Review Proceedings*

This appeal arises from the IPR process enacted through the Leahy-Smith America Invents Act, Pub. L. No. 112-29 (2011) ("AIA"). In the AIA, "Congress established a process for *inter partes* review of an issued patent within the PTO." *St. Jude Med. v. Volcano Corp.*, 749 F.3d 1373, 1374 (Fed. Cir. 2014). Under that process, any person other than the patent owner may petition the Patent and Trademark Office ("PTO") to initiate an IPR. 35 U.S.C. § 311; *see also id.* §§ 312, 313 (describing the required contents of an IPR petition and allowing a response by the patent owner).

Within three months of receiving a response to the petition (or, if there is no response, three months after the time to respond has expired), the PTO Director "shall determine whether to institute an *inter partes* review." 35 U.S.C. § 314(b). The Director may not institute an IPR unless "there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged." *Id.* § 314(a). And the statute mandates that "[t]he determination by the Director whether to institute an *inter partes* review . . . shall be final and nonappealable." *Id.* § 314(d). That provision "prohibits review of the decision to institute IPR even after a final decision." *In re Cuozzo Speed Techs. LLC*, __ F.3d __, 2015 WL 4097949, at *3 (Fed. Cir. 2015).

The Director has delegated to the Board the authority to consider IPR petitions. 37 C.F.R. § 42.4(a). If the Board decides to institute an IPR, it issues an "institution decision," which "identif[ies], on a claim-by-claim basis, the grounds on which the trial will proceed." Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,765 (Aug. 14, 2012); *see* 35 U.S.C. § 314(c) (requiring that the Director "notify the petitioner and patent owner, in writing" of its decision to institute review). Under PTO regulations issued pursuant to the AIA, *see* 35 U.S.C. § 316, a patent owner's response to the institution decision may **only** address grounds "not already denied" by the Board. 37 C.F.R. § 42.120(a).

After an institution decision is issued, the IPR proceeding itself takes place before the Board, limited to the grounds accepted for review in the institution decision. *St. Jude Med.*, 749 F.3d at 1374. Congress structured IPRs as adversarial, trial-like proceedings. The AIA requires that the IPR must be open to the public; provides for "discovery of relevant evidence" and sanctions for discovery abuses; authorizes protective orders for submission of confidential materials; provides for a right to submit written evidence and obtain an oral hearing; and allows for joinder of parties. 35 U.S.C. §§ 315(c), 316(a)(1), (5)-(7), (9), (10), (12). The statute places on the petitioner "the burden of proving a proposition of unpatentability by a preponderance of the evidence." *Id.* § 316(e). It also provides that the Board's final decision must be in writing. *Id.* § 318(a).

And any such final decision has preclusive effect as to both future PTO proceedings and judicial proceedings, as to "any ground that the petitioner raised or reasonably could have raised during [the] *inter partes* review." *Id.* § 315(e).

Either party may appeal an adverse final decision directly to this Court. *Id.* § 319. This Court's review is "on the record before the Patent and Trademark Office." *Id.* § 144.

2. *The Board's Institution Decisions*

BioMarin filed two petitions requesting *inter partes* review of claim 1 of the '410 patent (the "'410 petition") and claims 1 and 3-6 of the '226 patent (the "'226 petition").

Regarding the '410 patent, the Board instituted *inter partes* review on two grounds, both of which asserted invalidity under 35 U.S.C. § 103. The first ground was based on the combination of Reuser (Ex 1005) [A914-53], Barton (Ex 1004) [A907-13], and Van der Ploeg (Ex 1032) [A1262-66], and the second on the combination of the "Duke Press Release" (Ex 1002) [A901-02], Barton (Ex 1004 [A907-13], and Van der Ploeg (Ex 1032) [A1262-66]. A167. The Board **denied** as redundant the five remaining proposed grounds set forth in BioMarin's petition, including one involving, among other references, Kikuchi (Ex 1007) [A963-69]. *Id.*

The Board instituted *inter partes* review as to the '226 patent only with

respect to one proposed ground for each set of claims, namely, (i) alleged obviousness of claims 1 and 3 over the combination of the Duke Press Release (Ex 1002) [A901-02], Reuser (Ex 1005) [A914-53], and Van Hove (Ex 1012) [A997-1007], and (ii) alleged obviousness of claims 4-6 over the combination of the Duke Press Release (Ex 1002) [A901-02], Reuser (Ex 1005) [A914-53], Barton (Ex 1004) [A907-13], and Van der Ploeg (Ex 1032) [A1262-66]. A6838. The Board denied BioMarin's anticipation claim and denied as redundant the remaining proposed obviousness grounds. A6839-40.

The Board's initial constructions of claims 1 and 6 (the two independent claims) of the '226 patent and claim 1 of the '410 patent included a determination that the results set forth in the "whereby" clauses of those claims were not separate, patentable features, citing precedent such as *Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc.*, 246 F.3d 1368, 1376 (Fed. Cir. 2001). A6834-35.

BioMarin did not seek reconsideration of either institution decision.

3. *The Board's Final Written Decisions*

In its final written decisions, unlike in its institution decisions, the Board concluded that the whereby clauses of the claims were material to patentability. The Board for the first time construed those clauses, and because the alleged basis for invalidity was obviousness, a critical question the Board was required to—and did—answer was whether a person of ordinary skill in the art ("POSA") would

have had a reasonable expectation of successfully achieving the results set forth in the whereby clauses. A33-36; A4-7. Because that question was not considered in the institution decisions—again, those decisions did not consider the critical whereby clauses—the legal analysis in the final written decisions looked nothing like the analysis in the institution decisions. And because the Board conducted an entirely new reasonable-expectation-of-success analysis, the factual analysis of the scope and content of the prior art in the final written decisions was also entirely different than that in the institution decisions, as detailed below. *See infra* Part II.

Under this new legal and factual analysis, the Board concluded that the '410 and '226 patents were invalid under 35 U.S.C. § 103. A11-22; A40-51. The Board's obviousness findings were based in large part (A14-15; A41) on a conclusion that the claims did not necessarily include reducing or arresting glycogen buildup in skeletal muscle, but could cover reducing or arresting glycogen buildup in liver **alone**, A6-7; A35-36, even though it was well understood at the time of the invention that such a result does not constitute treatment of human Pompe disease. A15-18; A41-44; *see supra* at 6-8.

Nowhere in the final decisions did the Board make a specific factual finding regarding the level of ordinary skill in the art at the time of the invention. *See infra* Part IV.A.

SUMMARY OF ARGUMENT

The Board's final decisions should be reversed—and judgment entered in favor of Genzyme—for three independent reasons.

First, the Board violated the notice provisions of the Administrative Procedure Act ("APA") because its final decisions relied almost entirely on legal and factual analysis not noticed in the institution decisions.

IPRs are trial-like procedures subject to the protections afforded in formal adjudications under the APA, including the notice requirements set forth in 5 U.S.C. §§ 554(b)(3) and 557(c). The Board's institution decisions set forth the legal and factual bases for a potential finding of invalidity and, thus, put Genzyme on notice of the legal and factual arguments it would be required to meet. Yet the Board's final written decisions bear little resemblance to the institution decisions. For example, the Board's obviousness analysis in the institution decisions completely ignored the claims' whereby clauses, instead concluding that the claims may be invalid because the glycogen reduction or arrest described in the whereby clauses was not a separate, patentable feature. The final decisions, in contrast, made no mention of that argument, instead holding that the whereby clauses must be considered, construing those clauses, and concluding that the claims were obvious based on that construction and a reasonable-expectation-of-success analysis. In other words, the Board's final decisions were based on a legal theory

found nowhere in the institution decisions. So too with the Board's factual analysis—the Board's final decisions are based on entirely new facts from: (i) references that were not cited in the institution decisions, and (ii) references that were cited, but for entirely different propositions. This dramatic shift in the factual and legal bases for the Board's final decisions flatly violates the notice provisions of the APA. And because the Board could not possibly cure that notice problem in a manner that would lead to a legally sustainable finding of unpatentability, setting aside the final written decisions should result in a final decision upholding the patentability of the challenged claims.

Second, the Board's finding that the claims are invalid on obviousness grounds was based on its erroneous construction of the whereby clauses. The patents claim a "method of treating a human patient with Pompe's disease" through the administration of GAA "**whereby** the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested." The Board construed the whereby clause to include reducing or arresting glycogen buildup in liver alone (and, in turn, held the claims obvious because a POSA would have had a reasonable expectation of success in achieving that result).

The Board's construction is fundamentally flawed. For one thing, all agree that at the time of the invention, it was understood that Pompe disease **could not**

be treated by reducing or arresting glycogen buildup in liver alone, and that an effective treatment **must** achieve that result in (at a minimum) skeletal muscle. It is thus unreasonable to construe a claimed "method of **treating** a human patient with Pompe's disease" in a manner that everyone at the time understood could not possibly constitute treatment of a human patient with Pompe's disease. Both the specification and the prosecution history make clear that the claim would have been unambiguously understood, at a minimum, to require reducing or arresting glycogen buildup **in skeletal muscle**—a result the Board's own analysis shows could not have been reasonably expected by a POSA at the time of the invention.

Third, regardless of the proper claim construction, the Board's reasonable expectation of success finding must be reversed as unsupported by substantial evidence. As an initial matter, the Board failed to make a finding under *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966), concerning the level of ordinary skill in the pertinent art. And BioMarin—which bears the burden of proving invalidity—failed to offer competent, objective evidence of the state of knowledge at the time of the invention from the perspective of a POSA. To the contrary, BioMarin's expert admitted to conducting a **subjective and hindsight-infected analysis** of the prior art, an impermissible basis for an obviousness finding. Accordingly, the Board could only have found a reasonable expectation of success by using **its own** expertise, contrary to both the statutory burden of

proof and the settled principle, set forth in precedents of this Court, forbidding the Board from relying on its own expertise in contested proceedings like IPRs.

ARGUMENT

I. STANDARDS OF REVIEW

Board decisions are reviewed under the standards of review set forth in the APA. *In re Chapman*, 595 F.3d 1330, 1336-37 (Fed. Cir. 2010). The Board's legal conclusions are accordingly reviewed *de novo*, and its factual findings are reviewed for substantial evidence. *Id*.; *see* 5 U.S.C. § 706(2); *see also Rambus Inc. v. Rea*, 731 F.3d 1248, 1251 (Fed. Cir. 2013). Here, the Board's claim construction is reviewed *de novo* as it relied only on intrinsic evidence. *Microsoft Corp. v. Proxyconn, Inc.*, 789 F.3d 1292, 1297 (Fed. Cir. 2015); *In re Cuozzo*, 2015 WL 4097949, at *8-9. The Board's obviousness findings, which were based on its reasonable-expectation-of-success conclusion, are also reviewed *de novo*. Any factual findings supporting that conclusion are reviewed for substantial evidence. And because the petitioner in an IPR proceeding has the burden of proving unpatentability by a preponderance of the evidence, 35 U.S.C. § 316(e), the ultimate question is whether there was "substantial evidence" to conclude that BioMarin carried its burden. *See* 5 U.S.C. § 706(2)(E).

This case also presents the question whether the Board complied with the APA itself, i.e., whether the Board provided adequate notice of all material

"matters of fact and law asserted" in its institution decisions, 5 U.S.C. §§ 554(b)(3), 557(c)(3), or instead improperly "relied on a new ground of rejection" in its final decisions in violation of the APA. *In re Biedermann*, 733 F.3d 329, 335 (Fed. Cir. 2013) (quoting *In re Stepan Co.*, 660 F.3d 1341,1343 (Fed. Cir. 2011)). That "is a legal question that [this Court] review[s] *de novo*." *Id.* (quoting *In re Stepan Co.*, 660 F.3d at 1343).

II. THE BOARD VIOLATED THE APA'S NOTICE REQUIREMENTS BY RELYING ON NEW LEGAL AND FACTUAL GROUNDS IN ITS FINAL WRITTEN DECISIONS NOT NOTICED IN THE INSTITUTION DECISIONS

A. IPRs Are Formal Agency Adjudications Subject To The APA's Notice Requirements

The APA "governs the proceedings of administrative agencies and related judicial review." *Allentown Mack Sales & Serv., Inc. v. NLRB*, 522 U.S. 359, 374 (1998). The PTO "is an 'agency' subject to the APA's constraints." *Dickinson v. Zurko*, 527 U.S. 150, 154 (1999); *see In re Biedermann*, 733 F.3d at 336; *In re Sang Su Lee*, 277 F.3d 1338, 1342 (Fed. Cir. 2002).

Every "agency action"—any "rule, order, license, sanction, relief, or the equivalent or denial thereof, or failure to act"—is the product of an "agency proceeding," the minimum procedures for which are set forth in the APA. *See* 5 U.S.C. § 551(13). There are two general types of agency proceedings: rule makings and adjudications. The Supreme Court has described the "basic

distinction between rulemaking and adjudication" as a difference between "proceedings for the purpose of promulgating policy-type rules or standards, on the one hand, and proceedings designed to adjudicate disputed facts in particular cases on the other." *United States v. Fla. E. Coast Ry. Co.*, 410 U.S. 224, 245 (1973). As this Court has recognized, IPRs are adjudications. *See In re Cuozzo*, 2015 WL 4097949, at *7; *see also* H.R. Rep. No. 112-98, at 46-47 (2011) (AIA "converts *inter partes* reexamination from an examinational to an adjudicative proceeding").

The APA further distinguishes between formal and informal adjudications. "If an agency adjudication is 'required by statute to be determined on the record after opportunity for an agency hearing,' the adjudication becomes a formal proceeding." *Brand v. Miller*, 487 F.3d 862, 867 (Fed. Cir. 2007) (quoting 5 U.S.C. § 554(a)); *see Walls v. United States*, 582 F.3d 1358, 1377 (Fed. Cir. 2009). Under this Court's precedent, "an agency adjudication is deemed formal under the APA . . . when the agency's authorizing statute requires a hearing with trial-type procedures." *Walls*, 582 F.3d at 1377 (citation omitted).

IPRs easily satisfy that standard—they are adversarial, trial-like procedures, and are accordingly formal adjudications under the APA. Parties to an IPR are statutorily entitled to a hearing. 35 U.S.C. § 316(a)(10). And, as explained earlier, IPRs employ trial-type procedures, including discovery, submission of evidence, the use of protective orders, and joinder of parties. *See supra* at 9-11; *see also*

Patent Trial Practice Guide, 77 Fed. Reg. 48,756 (Aug. 14, 2012). IPRs also have preclusive effect just like any trial—in an IPR "that results in a final written decision," the petitioner is estopped from seeking to invalidate the patent in any new proceeding before the PTO **or in court** "on any ground that the petitioner raised or reasonably could have raised during [the] *inter partes* review." 35 U.S.C. § 315(e). These provisions amply demonstrate Congress's intent that IPRs would mirror judicial proceedings, both as to the formality and adversarial nature of the procedures as well as to the preclusive effect of the Board's decisions.

Congress and the Board itself understood that IPRs were, in effect, trials. Congress changed the Board's name from the "Patent Board of Appeals and Interferences" to the "Patent **Trial** and Appeal Board." 35 U.S.C. § 6 (emphasis added). And the PTO's own regulations state that "[a]n *inter partes* review **is a trial** subject to the procedures set forth" elsewhere in the regulations. 37 C.F.R. § 42.100(a) (emphasis added); *see also* 37 C.F.R. § 42.2 (including IPRs in PTO definition of "trial" before the Board). Finally, Congress required this Court to review a final decision in an IPR "**on the record** before the [PTO]." 35 U.S.C. § 144 (emphasis added). And "a requirement that administrative decisions be 'on the record'"—i.e., that the process leading to those decisions count as formal adjudication under the APA—"can be 'clearly implied in the provision for judicial review . . . in the circuit courts of appeal.'" *Marathon Oil Co. v. EPA*, 564 F.2d

1253, 1263 (9th Cir. 1977) (quoting Attorney General's Manual on the APA, at 41 (1947)).

The APA sets forth an array of trial-type protections that must be employed in all formal adjudications. *See* 5 U.S.C. §§ 554, 556, 557. Most relevant here, the agency conducting a formal adjudication **must** give notice of **all** material legal and factual issues to be addressed in the proceeding. Specifically, § 554(b) provides that "[p]ersons entitled to notice of an agency hearing shall be timely informed of—(1) the time, place, and nature of the hearing; (2) the legal authority and jurisdiction under which the hearing is to be held; and (3) **the matters of fact and law asserted**." 5 U.S.C. § 554(b) (emphasis added); *see In re Stepan Co.*, 660 F.3d at 1345. Section 557, meanwhile, mandates that "**[a]ll decisions, including initial, recommended, and tentative decisions**, . . . shall include a statement of . . . findings and conclusions, and the reasons or basis therefor, on **all** the material issues of fact, law, or discretion presented on the record." *Id*. § 557(c)(3)(A) (emphasis added).

B. The Board's Institution Decisions Provide The Notice Required By APA §§ 554(b) And 557(c)(3), And Those Provisions Preclude The Board's Final Decisions From Relying On New Grounds

As this Court has recognized when addressing appeals from examiner rejections and reexaminations, the APA requires the PTO to "provide prior notice to the applicant of **all** 'matters of fact and law asserted' prior to an appeal hearing

before the Board." *In re Stepan Co.*, 660 F.3d at 1345 (emphasis added) (quoting 5 U.S.C. § 554(b)(3)); *see also In re Leithem*, 661 F.3d 1316, 1319 (Fed. Cir. 2011). The APA demands no less of the PTO in IPRs. Consistent with that requirement, the Board's institution decisions must provide the parties with notice of all matters of law and fact asserted in an IPR before it moves forward to the hearing stage. 5 U.S.C. §§ 554(b), 557(c)(3).

IPRs proceed in two steps. *See St. Jude Med.*, 749 F.3d at 1375-76. In the first phase, the Board decides whether to institute a review, determining whether the information in the petition, along with any preliminary patent owner response, "shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a). The Board then issues an institution decision, in which it "identif[ies], on a claim-by-claim basis, the grounds on which the trial will proceed." 77 Fed. Reg. at 48,765; *see also* 35 U.S.C. § 314(c) (PTO must "notify the petitioner and patent owner, in writing" of its decision to institute review). "Any claim or issue not included in the authorization for review is not part of the trial," 77 Fed. Reg. at 48,765, and after a review is instituted, a patent owner may file a response **only** to address grounds "not already denied" by the Board, 37 C.F.R. § 42.120(a). The Board's institution decision thus sets the stage for the second phase of an IPR, identifying the issues to be heard and determined in a trial conducted by the Board.

37 C.F.R. § 42.2 ("A trial **begins** with a written decision notifying the petitioner and patent owner of the institution of the trial." (emphasis added)); 37 C.F.R. § 42.4(b) ("The entry of the notice institutes the trial."); A168 ("FURTHER ORDERED that all other grounds presented in the Petition are *denied*, and **no ground other than those specifically granted above is authorized** for the [IPR]" (emphasis added)); A6841 (same).

In appeals from examiner rejections and reexamination proceedings, this Court has warned that "[a]llowing the Board unfettered discretion to designate a new ground of rejection—**when it relies upon facts or legal argument not addressed by the examiner**—would frustrate the notice requirements of the APA." *In re Stepan Co.*, 660 F.3d at 1345. Thus, "[t]he ultimate criterion is whether the appellant has had before the PTO a 'fair opportunity to react to the thrust of the rejection.'" *Rambus*, 731 F.3d at 1255 (quoting *In re Jung*, 637 F.3d 1356, 1365 (Fed. Cir. 2011)). If the Board does not provide the appellant with an opportunity to respond, it "violates the appellant's notice rights" and its decision must be vacated. *Id.* at 1256 (citing *In re Stepan Co.*, 660 F.3d at 1346).

The same standards apply to IPRs. A patent owner is entitled to notice of the issues to be considered by the Board and an opportunity to address the facts and legal arguments on which the Board's patentability determination will rest. Indeed, in an IPR, unlike an appeal from an examiner rejection, the patent owner's

property rights have already vested—i.e., the question is not whether a patent should issue, but whether a patent that has **already issued** should be cancelled. The reasons for requiring the type of robust notice this Court has demanded of the PTO pursuant to the APA in other contexts are thus, if anything, even stronger in the IPR context.

C. The Board's Final Written Decisions Relied On New Grounds In Violation Of The APA's Notice Requirements

Consistent with the APA and the procedures that govern IPRs, the Board's institution decisions here noticed all of the law and facts on which the review was to proceed. Both institution decisions laid out the Board's construction of the challenged claims, set forth the specific legal and factual grounds of unpatentability to be considered, and cited the prior art references on which those grounds were based. A159-69; A6830-42. The Board's final written decisions, however, bear little resemblance to its institution decisions on either the law or the facts. The grounds upon which the Board found the challenged claims unpatentable in its final written decisions are significantly different than those set forth in the institution decisions, thus violating the APA's notice requirements. Those final decisions therefore must be set aside. *See* 5 U.S.C. § 706(2)(D).

To begin, the central feature of the Board's institution decisions was its conclusion, when construing the challenged claims of the '410 and '226 patents, that the reduction or arrest of glycogen called for by the whereby clauses was not a

separate, patentable feature, but merely a result of the processes described. A163-64 (citing *Abbott Labs. v. Baxter Pharm. Prods., Inc.*, 471 F.3d 1363, 1369 (Fed. Cir. 2006); *Bristol-Myers Squibb Co.*, 246 F.3d at 1376); A6834-35 (same). A party seeking to invalidate a patent as obvious must demonstrate "that a skilled artisan would have had reason to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success from doing so." *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1069 (Fed. Cir. 2012). But as a consequence of the Board's claim construction in its institution decisions—i.e., its determination that the whereby clauses were not relevant to patentability—those decisions did not address whether, based on the references underlying the grounds granted, a POSA would have had a reasonable expectation of success in using GAA to reduce or arrest glycogen buildup in a patient with Pompe disease. The institution decisions were accordingly also devoid of any corresponding discussion of data and experimental results that might support such a reasonable-expectation-of-success finding.

In its final written decisions, the Board changed course, recognizing that the whereby clauses, which require a reduction or arrest of glycogen buildup, **are** material to patentability, and thus must be construed and considered when assessing obviousness. *See* A5-7; A33-36. The Board then conducted the

reasonable-expectation-of-success inquiry, concluding that "a person of ordinary skill in the art would have had a reasonable expectation of success at the time the invention was made."[2] A17-18; A43. The Board's final written decisions contain no reference to *Bristol-Myers Squibb Co.* or *Abbott Labs.*—the cases at the core of its legal analysis in the institution decisions—or to the underlying "result of the process described" principle for which those cases stand, confirming the dramatic shift in the Board's position. Given the institution decisions, the Board failed to provide Genzyme notice that a construction of the whereby clauses and a likelihood-of-success analysis would be at issue at all, let alone central to the Board's analysis. That lack of notice violates the APA. *See Rambus*, 731 F.3d at 1255.

Compounding the problem, the Board's conclusion that there was a reasonable expectation of success was based on a factual analysis materially different from that found in its institution decisions. The Board relied on new prior art references not cited in the grounds of the institution decisions, and on new teachings from prior art references that were cited in the institution decisions for

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[2] All the challenged claims contain whereby clauses referring to a reduction or arrest of glycogen buildup except for claim 6 of the '226 patent, which references a reduction or arrest of hypertrophic cardiomyopathy. But the Board did not present independent reasoning as to claim 6 of the '226 patent, instead "conclude[ing] that independent claim 6 is unpatentable for the reasons set forth above with regard to claim 1." A49.

completely different purposes. Together, these new additional facts clearly changed the scope and content of the teachings of the prior art and in effect presented a new and different legal question regarding obviousness. *See In re Rouffet*, 149 F.3d 1350, 1355 (Fed. Cir. 1998) (ultimate determination of obviousness is a question of law).

For example, the Board's final written decisions cite two prior art references that were not mentioned in the grounds set forth in the institution decisions: Kikuchi (Ex 1007) [A963-69] and Van der Ploeg (Ex 1009) [A977-82]. Van der Ploeg, in fact, appears **nowhere** in either institution decision. Kikuchi, meanwhile, is not mentioned in the '226 patent institution decision at all, and the Board referenced Kikuchi in the '410 patent institution decision only in connection with its **denial** of the single asserted ground that included that reference, concluding that the ground that included Kikuchi should be denied as "redundant." A166-67.

Both Kikuchi and Van der Ploeg are sources of *in vivo* data regarding GAA's effects. In the final written decisions, the Board cited Kikuchi for "data suggesting that the intravenous administration of human GAA to GAA-deficient Japanese [quail] reduced glycogen levels in the heart, liver and muscle and produced muscle improvement," A16; A42, and noted that its authors had concluded that the data "suggest[ed] enzyme replacement with recombinant human GAA is a promising therapy for human Pompe disease," A16 (quoting A963); A42

(same). Similarly, in the final written decisions, the Board cited Van der Ploeg as a source of "data suggesting that GAA containing mannose 6-phosphate is taken up in the skeletal muscle and heart of mice after intravenous administration." A16; A42. Not only did the Board's institution decisions fail to include either of these two references in the grounds for review (indeed, one was expressly declared to be outside the scope of the trial grounds), but the institution decisions **did not mention *in vivo* data at all**. The Board's final written decisions thus relied on references and data reflecting an entirely different analysis of the scope and content of the prior art than that set forth in the institution decisions.

As this Court has explained, citation of a new reference amounts to a new ground of rejection—to which the patentee must be afforded an opportunity to respond—unless the reference "is a standard work, cited only to support a fact judicially noticed **and** . . . the fact so noticed plays a minor role." *In re Biedermann*, 733 F.3d at 338 (emphasis added; quotation omitted). Neither of the new prior art references cited by the Board is even purported to be a "standard work," nor were they cited to support a judicially noticed fact, let alone one that played only a minor role in the Board's reasoning. Instead, both were cited in support of the Board's characterization of the state of "the field related to the development of an enzyme replacement therapy for the treatment of Pompe disease" as of December 7, 1998, and both were used by the Board to support its

reasonable-expectation-of-success finding. A8-9; A16-17; A37; A42-43 ("**In view of the above**, we conclude that a [POSA] would have had a reasonable expectation of success . . . ." (emphasis added)). The new references in the Board's final written decisions, which were crucial to the Board's finding of unpatentability, thus constitute new grounds, and the Board violated Genzyme's notice rights by relying on them without prior notice. *See In re Stepan Co.*, 660 F.3d at 1346.

The Board's final written decisions also ran afoul of the APA's notice requirements by relying on new facts extracted from previously cited prior art references. *See In re Biedermann*, 733 F.3d at 337 ("[M]ere reliance on the same statutory basis and the same prior art references, alone, is insufficient to avoid making a new ground of rejection when the Board relies on new facts and rationales." (citing *In re Leithem*, 661 F.3d at 1319)); *In re Kumar*, 418 F.3d 1361, 1367-68 (Fed. Cir. 2005) (same).

First, the Board's final written decisions quoted an entirely new passage from Reuser (Ex 1005) [A914-53] describing studies of muscle tissue uptake where GAA was administered to mice. A8; A37. The quoted passage in turn cites two additional prior art references found nowhere in the Board's institution decisions: Van der Ploeg (Ex 1009) [A977-82], discussed above, and Van der Ploeg (Ex 1051) [A1474-77]. *See* A8; A37. Second, while Van der Ploeg (Ex 1032) [A1262-66] was cited in the institution decisions, with respect to the claimed

dosing schedules, as a source of information relating to the **half-life** of GAA, A165, A6838, the final written decisions relied on that reference, as part of the Board's **reasonable-expectation-of-success finding**, for data on ***in vitro* uptake of GAA in skeletal muscle cells**, concluding that the reference demonstrated "the concept of using human GAA to treat Pompe disease was known in the art." A19-21; *see* A47. Third, in its final written decisions, the Board cited what it described as a "Duke Press Release" (Ex 1002) [A901-02], to show that "the FDA was granting applications for orphan drug designation for enzyme replacement therapy for Pompe disease using recombinant GAA." A16; A42. The Board further noted that "the FDA [orphan drug designation] application process requires an applicant to provide 'enough information to establish a medically plausible basis for expecting the drug to be effective in the rare disease.'" A16; A42 (citing Ex 1029 [A1192-95], another reference not mentioned in the institution decisions). Again, the Board departed from its institution decisions, where it described the "Duke Press Release" only as "detail[ing] an FDA clinical trial study in which infants with Pompe's disease were injected with recombinant [GAA] in order to evaluate the safety and efficacy of the recombinant enzyme treatment." A164; A6836. Setting aside the fact that the Board was incorrect (as it ultimately acknowledged) in characterizing the clinical trial described in that reference as having been initiated, the institution decisions contained no mention of the FDA's grant of an

orphan drug designation or its implications.

The Board's reliance on entirely new legal and factual grounds in its final decisions requires reversal under the APA.

D. The Board's Approach Is At Odds With The Goals Of The IPR Process And The Procedures Put In Place To Advance Them

This case demonstrates the importance of assuring that the Board complies with the APA's procedural requirements. Permitting the Board freely to depart from its institution decisions—as it has done here—would place an unfair burden on patent owners like Genzyme and would defeat the goals of the IPR process. Patent owners would not be able to rely on the notice provided in the Board's institution decisions, but would instead be required to anticipate what new prior art references the Board might rely on, what facts the Board would extract from those references, and how the Board would combine them, and then formulate a response to all such potential references and combinations. This would force patent owners to respond comprehensively to **all** references cited in the petition, regardless of how the Board's institution decision narrowed the grounds on which the IPR was to proceed. Here, BioMarin filed **over one hundred exhibits** in connection with each of its petitions, the vast majority of which were publications dated prior to December 1998. If patent owners were required to address each reference cited in a petition, the scale of the IPR proceeding would expand dramatically, and the Board's institution decision would no longer do anything to "streamline" the

review process. 77 Fed. Reg. at 48,765; *see* 37 C.F.R. § 42.1(b) (stating that regulations governing proceedings before the Board "shall be construed to secure the just, speedy, and inexpensive resolution of every proceeding").

As one example, had Genzyme known that the Board would ultimately rely on Kikuchi (Ex 1007) [A963-69]—or any of the other five § 102(a) prior art references mentioned in BioMarin's petitions—even though it did not do so in the institution decisions, Genzyme would have pursued the option of establishing that it invented the patented methods of treatment before each of the § 102(a) references was published.[3] But to do so, Genzyme would have had to expend significant resources associated with the submission of additional evidence, declarations, and further depositions.

To take another example, if the Board were allowed to rely on prior art mentioned only in grounds denied in its institution decisions, as it did here, there would seemingly be nothing to stop the Board from likewise revisiting its decision on anticipation—an additional ground asserted by BioMarin but denied by the Board. *See* A6835-36; A6841. Genzyme would therefore be compelled to

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[3] BioMarin's petitions argued that, along with Kikuchi (Ex 1007) [A963-69], several other references were "also relevant" because "the inventors . . . have not established an invention date before December 7, 1998." A102 (citing Exhibits 1014 [A1012-21], 1031 [A1255-61], 1116 [A2673], 1076 [A2262-71], 1072 [A2221-22]); A6767-68 (same).

preemptively address that separate legal issue as well.

This emphatically is **not** how IPRs are intended to work. Under the regulations, a patent owner is not even **allowed** to address asserted grounds of invalidity that have been denied by the Board. 37 C.F.R. § 42.120(a). Moreover, unlike with amending claims during an IPR, where the burden is on patent owners to distinguish proposed substitute claims over all prior art of record, *Proxyconn*, 789 F.3d at 1308, the burden to demonstrate invalidity here unquestionably lies squarely on BioMarin. 35 U.S.C. §§ 312, 316; *see* 37 C.F.R. §§ 42.20(c), 42.22(a)(2). If BioMarin was dissatisfied with the Board's institution decisions, it could have—and should have—sought rehearing. 37 C.F.R. § 42.71(d). But when BioMarin did not do so, the IPR went forward on the understanding, reflected in the PTO's own regulations, Patent Trial Practice Guide, and institution decisions, that any matters not included in the Board's institution decisions were not part of the proceeding. The Board's refusal to confine its final written decisions to the facts and law set forth in its institution decisions deprived Genzyme of the notice required by the APA. As a result, the Board's final written decisions must be vacated. *Rambus*, 731 F.3d at 1256 (citing *In re Stepan Co.*, 660 F.3d at 1346).

E. There Is No Basis For A Remand To The Board

Vacating the Board's final written decisions must result in a denial of BioMarin's challenges, as the lack of notice to Genzyme cannot be cured.

As this Court has made clear, institution decisions are final and cannot be modified, 35 U.S.C. § 314(d); *In re Cuozzo*, 2015 WL 4097949, at *3, and there is no mechanism in the statute or governing regulations for altering an institution decision once it has been made and a final written decision has issued. Because the institution decisions serve as the APA-required notice, any final written decision of the Board in this case must not deviate from the **existing** institution decisions in a manner that would run afoul of the APA requirements discussed above.

There is, however, no possible final written decision the Board could issue here that could be sufficiently similar to its institution decisions to satisfy the APA's notice requirements while at the same time legally supporting unpatentability. As explained below, the Board's final written decisions **correctly** concluded that the patents' whereby clauses are material to patentability. *See infra* Part III. But the Board in its institution decisions neither construed nor conducted a reasonable-expectation-of-success analysis with respect to those clauses. *See supra* Part II.C. Since those analyses are prerequisites to any obviousness finding, any final written decision that resembles the initiation decisions—i.e., that does not construe or contain a reasonable-expectation-of-success analysis as to the whereby clauses—would fail as a matter of law. *In re Cyclobenzaprine Hydrochloride*, 676 F.3d at 1069-70; *supra* Part II.C.

Indeed, were there any sound basis to invalidate the patents on the grounds stated in the institution decisions, the Board presumably would have adopted those grounds in its final written decisions. The Board's decision to significantly deviate from its institution decisions is understandable given that the grounds stated therein could not support a finding of unpatentability. But because any final written decision that satisfies the APA's notice requirements could not as a matter of law result in a finding of unpatentability, there is no basis for remand to the Board. BioMarin's challenge to the patents must be finally rejected.

III. THE BOARD'S CLAIM CONSTRUCTION WAS ERRONEOUS

The Board's final decisions should also be reversed because they are predicated on incorrect claim constructions. The focus of disagreement centers on the patents' whereby clauses, which claim a "method of treating a human patient with Pompe's disease" through intravenous administration of "human [GAA], **whereby the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested**." A899; A1642 (emphasis added).

The Board **correctly** recognized in its final written decisions—unlike in its institution decisions—that the whereby clauses are essential to practicing the claimed methods of treatment and are thus material to patentability; much of the final written decisions is directed to assessing patentability in light of the whereby

clauses. *E.g.*, A5; A7-8; A12-16; A19-20; A32-36; A40-42; A45-47; A49; *see also Hoffer v. Microsoft Corp.*, 405 F.3d 1326, 1329 (Fed. Cir. 2005) ("[W]hen the 'whereby' clause states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention."). Moreover, the Board correctly recognized that the glycogen referenced in the claims must be **lysosomal** glycogen, even though that term is not explicitly set forth in the claims, since it is undisputed that Pompe disease is a lysosomal storage disease in which glycogen accumulates in the lysosomes of affected tissues. A7; A36. And the Board also correctly recognized that the claims "require the treatment of Pompe disease." A7.

But the Board clearly **erred** in construing the phrase "glycogen in a patient" to encompass lysosomal glycogen in the **skeletal muscle, heart, *or* liver**. A7; A36. That construction erroneously encompasses the reduction or arrest of glycogen in liver **alone**.

The Board's construction of the challenged claims to allow for glycogen reduction in liver alone is not reasonable. "Even under the broadest reasonable interpretation, the Board's construction 'cannot be divorced from the specification and the record evidence' and 'must be consistent with the one that those **skilled in the art would reach**.'" *Proxyconn*, 789 F.3d at 1298 (emphasis added) (quoting *In re NTP, Inc.*, 654 F.3d 1279, 1288 (Fed. Cir. 2011); *In re Cortright*, 165 F.3d 1353, 1358 (Fed. Cir. 1999)); *see In re Suitco Surface, Inc.*, 603 F.3d 1255, 1260

(Fed. Cir. 2010); *In re Buszard*, 504 F.3d 1364 (Fed. Cir. 2007).

Here, it is undisputed that as of the priority date, a POSA would have understood that the **only** effective "method of treating a human patient with Pompe's disease" would be one that reduces and prevents further accumulation of glycogen **in skeletal muscle and heart**, not in liver alone. *See supra* at 7-8. Common sense makes clear that the whereby clauses can only be reasonably read to claim a method of treatment that a POSA would have understood to be plausibly effective, i.e., a method that reduces and prevents further accumulation in the relevant target tissues, and not only in liver. Moreover, the Board's construction is inconsistent with the intrinsic evidence, its own factual findings about the patents' teachings, and the admissions of BioMarin's expert. As explained below, the intrinsic evidence as a whole compels a construction of "glycogen in the patient" that includes—at a minimum—lysosomal glycogen in skeletal muscle. Because the Board's construction does not require a reduction or arrest of accumulation of glycogen in skeletal muscle, and more broadly encompasses a reduction or arrest of accumulation of glycogen in liver alone, it is not a reasonable interpretation of the claims and must be reversed.

A. The Specification And Uncontroverted Facts Regarding The Nature Of Pompe Disease Compel A Construction That Requires Arrest Or Reduction Of Glycogen In Skeletal Muscle

The patent specification is "the single best guide to the meaning of a

disputed term" and is "[u]sually . . . dispositive." *Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1582 (Fed. Cir. 1996). "The construction that stays true to the claim language and most naturally aligns with the patent's description of the invention will be, in the end, the correct construction." *Renishaw PLC v. Marposs Societa' per Azioni*, 158 F.3d 1243, 1250 (Fed. Cir. 1998); *see also Honeywell Int'l, Inc. v. Universal Avionics Sys. Corp.*, 493 F.3d 1358, 1362 (Fed. Cir. 2007).

Here, it is undisputed that the claims at issue concern the **treatment** of Pompe disease. And the patent specification[4] compels a construction that recognizes two critical features of such treatment that would be understood by a POSA: (i) that Pompe disease is a genetic muscle disease characterized by the harmful accumulation of glycogen in the lysosomes of **skeletal muscle** (and **heart**, in infants), and (ii) that the goal of the treatment of a patient with Pompe disease is to reduce or arrest the accumulation of that glycogen. *See supra* at 3-8.

Common sense dictates that muscle be an essential target of treatment given that Pompe disease is indisputably a **genetic muscle disease**. A3-4; A3376 (BioMarin's expert agrees that "Pompe disease is a genetic muscle disease."). And Genzyme's construction addresses this fact in a straightforward, logical manner: according to the specification, there are two clinical forms of Pompe disease: (i)

[4] The specifications of the '410 and '226 patents are virtually identical.

early onset infantile and (ii) late onset juvenile and adult. Early onset infantile Pompe disease presents shortly after birth and is associated with **muscular weakness and cardiac failure**, while symptoms of late onset Pompe disease in juvenile and adult patients occur later in life and "**only skeletal muscles** are involved." A1629; A885 (emphasis added). The claims cover patients with Pompe disease generally. The common denominator among both clinical forms of Pompe disease is glycogen accumulation in skeletal muscle. Thus, any reasonable construction of the claims must **at a minimum** encompass at least the treatment of muscle tissue.

Indeed, the specification explains—and the Board found—that "[t]he deficiency in the lysosomal enzyme **results in harmful accumulation of glycogen in muscle**. *Id*. at col. 15, ll. 26-29." A3-4 (emphasis added); *see also* A885 ("Infantile GSD II . . . presents with progressive **muscular weakness** and **cardiac failure**. . . . Symptoms in the late onset in adult and juvenile patients occur later in life, and **only skeletal muscles are involved**." (emphasis added)); A892. And the Board also correctly recognized that the claims, read in light of the specification, are directed to arresting or reducing glycogen levels in skeletal muscle: "[t]he **claimed treatment results** in the **arrest or reduction** of clinical and biochemical characteristics of Pompe disease, which include, generally, an **accumulation of glycogen** in various tissues **such as heart and skeletal muscle**, and more

specifically, **hypertrophic cardiomyopathy** [the subject of the whereby clause of claim 6 of the '226 Patent]." A32 (emphasis added); *see also* A3 (citing A891-92); A1635-36.

Furthermore, the specification includes several prophetic clinical trials that describe the administration of recombinant human GAA to patients with Pompe disease. In each of these examples, the parameters to be measured include those relevant to assessing whether there has been an arrest or reduction of the accumulation of glycogen in **skeletal muscle**. These include measurement of GAA activity in muscle, muscle histopathology, glycogen content in muscle, and muscle strength. A891-92; A1635-36; *see also* A1641; A897 (referencing measurement of "[g]lycogen content in muscle" and "[c]hanges in skeletal muscle [GAA] activity and glycogen content"). In these prophetic clinical trials, **no** mention is made of measuring efficacy in liver.

The specification's teachings are also consistent with the general knowledge at the time of invention, as conceded by BioMarin's expert Dr. Pastores. Dr. Pastores admitted during cross-examination that as of that date, it was understood "that the most prominent manifestation of Pompe disease across all subtypes was in skeletal muscle." A3274. He further admitted that "logically it flows that skeletal muscle **at a minimum**" was one of the organs persons would have looked to target in treating Pompe's disease. *Id.* (emphasis added). And he

testified that "[i]n treating Pompe disease one wants to target **both heart and skeletal muscle**." A1224; *see also* A4476.

Therefore, the "single best guide" to the meaning of the claims, read with knowledge of undisputed basic facts regarding the nature of Pompe disease, as confirmed by BioMarin's own expert, clearly supports a construction that requires—at a minimum—a reduction of glycogen in the lysosomes of **skeletal muscle**. *Vitronics*, 90 F.3d at 1582. A reduction in liver alone simply would not be effective to treat Pompe disease—the expressly stated objective of the patented methods.

B. The Board's Claim Construction Is Inconsistent With The Prosecution History As A Whole

The prosecution history confirms that the whereby clauses of claim 1 of the '410 and the '226 patents describe a method of treating Pompe disease by arresting or reducing glycogen buildup in skeletal muscle, not liver alone.

As an initial matter, the whereby clauses were added during prosecution of the application that ultimately issued as the '410 patent. A1111-20. As the remarks accompanying an amendment to the claims explain, the claims are supported by experimental data presented in the specification, including long-term animal experiments in an accepted knock-out mouse model of Pompe disease. *See* A1113; A1115-16. Those experiments make clear that effective treatment in mice was achieved by decreasing glycogen buildup **in skeletal muscle**. In particular,

Example 4, entitled "Animal Trial of Alpha-Glucosidase," reports the results of a study where GAA was administered to knock out mice. A1570-78. One group of mice in this example (the "Group C mice") was treated with GAA for 25 weeks. "[The Group C mice] showed **definite decrease in glycogen levels in liver, spleen, heart *and* skeletal muscle**." A1576 (emphasis added); *see also* A896; A1640. The '410 patent application also states that a "[c]lear reversal of pathology was demonstrated in various tissues, **such as heart and pectoralis muscle**." A1578 (emphasis added); *see also* A896; A1640.

More importantly, the applicants specifically explained that the data presented in the application supports the claims **and distinguishes the invention from the prior art**, including the de Barsy (Ex 1010) [A983-89] and Williams (Ex 1021) [A1102-10] references. Both of those references describe **failed** attempts to treat Pompe disease by administering GAA to humans, **notwithstanding that both studies reported uptake of enzyme into the *liver***. *See* A985; A1102; *see also* A977; A4480. The applicants explained that "neither de Barsy *et al*. nor Williams *et al.* describe continued administration of a therapeutically effective dose, such that the concentration of accumulated glycogen is reduced and/or the accumulation of glycogen is arrested, as set forth in the amended claims." A1116. "**In contrast** to these two references," they went on, "the present application has shown clear and significant reduction in glycogen content in a recognized mouse model for

Pompe disease." *Id.* (emphasis added). The applicants further noted that "[c]lear reversal of pathology has been demonstrated in various tissues, including **heart and pectoralis muscle**." A1115 (emphasis added; citing A1578). Based on this data, the applicants concluded: "**Thus,** Applicants have clearly demonstrated a therapeutic effect in an appropriate mammalian model of the disease." *Id.* (emphasis added).

Other references in the prosecution history also support a construction that requires reduction of glycogen—at a minimum—in skeletal muscle. For example, in an October 15, 2001 response, A1063, the applicants discussed recent clinical trial results that showed clinical improvement in muscle and heart, and noted "it was not clear from the cited art to what extent [GAA]" could be taken up by the "key target organs in a human when supplied exogenously." They went on to distinguish de Barsy (Ex 1010) [A983-89] on the basis that no uptake was detected "except in the liver," and similarly distinguished various other references cited by the examiner on the basis that they "discuss uptake of enzyme by cells *in vitro* or cite to the work of others concerning uptake of enzyme by **heart or liver** in a guinea pig. These results do not indicate the extent to which [GAA] will be taken up by the **heart or *muscle*** in a human infantile Pompe's disease patient." A1064 (emphasis added).

The prior art cited during prosecution of the '410 and '226 patents, which

recognizes that Pompe disease is a genetic muscle disease and that treatment of Pompe disease **must** target glycogen in the lysosomes of skeletal muscles, provides further intrinsic support for Genzyme's proposed construction. *See Powell v. Home Depot U.S.A., Inc.*, 663 F.3d 1221, 1230-31 (Fed. Cir. 2011) (explaining that references cited during prosecution constitute intrinsic evidence). For example, consistent with Genzyme's proposed construction, Van Hove describes the need to reduce glycogen levels in muscle and heart and explains that targeting liver alone is insufficient:

> Currently, there is no effective treatment for Pompe disease. **Initial enzyme replacement therapy trials using [GAA]** purified from human placenta normalized [GAA] and glycogen levels **in liver**, but not in muscle and heart of patients with Pompe disease. **To achieve therapeutic success, the administered enzyme *must* target the lysosomes of the affected organs, muscle and heart**.

A997 (emphasis added). BioMarin's expert admitted during cross-examination that this statement was both scientifically and factually accurate. A3401-02. Other prior art cited during prosecution also confirms the point. *See* A1265 ("By supplying high uptake forms of [GAA] **muscle** cells may compete with macrophages for enzyme capture" and "receptor-mediated enzyme replacement therapy for [Pompe disease] may be feasible, if high uptake forms of [GAA] would **gain sufficient access to affected muscle tissue**." (emphasis added; citation omitted)).

The Board completely ignored this extensive prosecution history and instead myopically focused on a single passage in it, taken out of context. In particular, the Board focused on the applicants' remarks that accompanied their May 30, 2006 Amendment. There, the applicants stated that "[s]upport for the amendments to Claim 1 can be found, **for example** . . . at p. 32, lines 1-2 and lines 31-33 (for reduction of accumulated glycogen and/or arrest of further accumulation of glycogen)." *See* A6; A34-35 (citing A1113) (emphasis added). The specific lines of the patent application cited as support for the proposed amendment describe the results of a subset of the experiments presented in the specification. At lines 1-2, the application describes the results of experiment B, stating: "[w]hen two [knock out] mice were injected 4 times every 6 days (experiment B), a marked decrease of total cellular glycogen was observed in both **heart *and* liver**." A1575 (emphasis added). At lines 31-33, the application describes experiment A, stating: "The results showed that mice treated 13 weeks with 0.5 mg/mouse (Group A, 3 animals/Group) had an increase of activity in the liver and spleen and decreased levels of **glycogen in liver *and* perhaps in heart**." A1575 (emphasis added).

These two sentences do not support the Board's own construction, which encompasses a reduction in liver glycogen **alone**. Both sentences refer to a reduction of glycogen in **liver *and* heart**. And as explained, the Board's construction is affirmatively precluded by the patent specification as a whole, and

the extensive prosecution history that it completely ignored, which (i) highlights successful experiments reducing glycogen levels in skeletal muscle, (ii) specifically contrasts the claims from prior art that disclosed reduction of glycogen in liver alone, and (iii) recognizes that the only effective method for treating Pompe disease is to reduce accumulation of glycogen in relevant target tissues, not liver alone. *See supra* at 41-45.

The prosecution history, viewed as a whole, thus refutes the Board's construction. By contrast, the prosecution history **is** consistent with Genzyme's proposed construction. Further, to the extent this Court finds that the prosecution history lacks clarity, the specification must guide the claim construction analysis. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1317 (Fed. Cir. 2005) (en banc) ("[B]ecause the prosecution history represents an ongoing negotiation between the PTO and the applicant, rather than the final product of that negotiation, it often lacks the clarity of the specification and thus is less useful for claim construction purposes." (citations omitted)); *see also Netcraft Corp. v. eBay, Inc.*, 549 F.3d 1394, 1401 (Fed. Cir. 2008) (finding prosecution history "lacks the clarity" and adopting construction based solely on specification). And the specification, as explained, clearly limits the claim to reduction—at a minimum—of glycogen in skeletal muscle, not in liver alone. *See supra* Part III.A.

In sum, a POSA taking into account the context of the claims (i.e., they are

method of **treatment** claims), the teachings of the specification, the intrinsic evidence as a whole, and fundamental general knowledge about Pompe disease would construe "concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested" to mean, at a minimum: "concentration of accumulated lysosomal glycogen in skeletal muscle of a human patient with Pompe disease is reduced and/or further accumulation of glycogen is arrested." The Board's contrary construction is wrong and must be rejected.

C. The Board's Flawed Claim Construction Requires Reversal Of Its Obviousness Findings

The Board's conclusion that the challenged claims in the '410 and '226 patents are invalid on grounds of obviousness cannot be sustained once its claim construction is corrected. In both final decisions, the Board rejected Genzyme's argument that a POSA "would not have had a reasonable expectation of successfully reducing or arresting further accumulation of glycogen in a patient's muscle cells." A40; A14. The Board specifically rejected this argument on the basis that it was "premised . . . on a claim construction that would require the phrase 'glycogen in the patient' in claim 1 necessarily to include lysosomal glycogen in skeletal muscles," and that the Board did "not adopt [Genzyme's] claim construction in this regard." A41; A14-15.

Indeed, had the Board adopted Genzyme's correct claim construction, a finding of obviousness would have been inconsistent with the Board's other

findings and the admissions of BioMarin's expert. Specifically, the Board recognized that no human clinical trials had yet been conducted at the time of the invention. A45; A12. The Board also recognized "that to treat Pompe disease **effectively** using GAA, sufficient quantities of enzyme would have to reach the patient's **muscle cells**, which could potentially require high doses that could introduce safety and efficacy hurdles resolvable **only** with human clinical trials." A45-46; *see* A12-13 (same). And BioMarin's expert Dr. Pastores conceded that there was **no known correlation** between GAA dose or dosing schedule and the reduction or arrest of further glycogen buildup. A3415-16.

Thus, had the Board correctly construed the claims to be limited—at a minimum—to reducing or arresting glycogen buildup in skeletal muscle, it also would have had to conclude that the **safety and efficacy profile** of treatment using GAA could not have been predicted as of the invention date. That would have precluded an obviousness finding because, as Judge Taranto has recently observed, when conducting a reasonable expectation of success analysis, "[t]he success that must be reasonably expected [would] have to be success in what motivated the investment in the research—**an acceptable safety/efficacy profile for human-therapeutic use**." *Bristol-Myers Squibb Co. v. Teva Pharms. USA, Inc.*, 769 F.3d 1339, 1355 (Fed. Cir. 2014) (Taranto, J., dissenting from denial of rehearing en banc) (emphasis added); *see Leo Pharm. Prods. Ltd. v. Rea*, 726 F.3d 1346, 1357

(Fed. Cir. 2013) ("[E]ven if it was obvious to experiment with these options 'there is nothing to indicate that a skilled artisan would have had a reasonable expectation that such an experiment would succeed in being **therapeutically effective**.'" (quoting *In re Cyclobenzaprine Hydrochloride*, 676 F.3d at 1070) (emphasis added)). Here, the Board's own fact-finding and BioMarin's expert's admissions demonstrate that a POSA at the time of the invention would have had no reason to expect that ERT involving GAA would be **therapeutically effective** in treating Pompe disease.

In its final written decisions the Board stated in a footnote that while "we do not interpret the claim to necessarily require the reduction or arrest of stored glycogen in skeletal muscle," "whether we include or exclude the result of reduction or arrest of stored glycogen in skeletal muscle within the scope of the claims, our conclusions remain the same." A46; A13. To the extent the Board meant to suggest that the challenged claims would be obvious even if (properly) construed to include reduction or arrest of glycogen in (at a minimum) skeletal muscle, that conclusion cannot be sustained for several reasons. To begin, any such finding would be wrong as a matter of law under the correct legal standard for the reasons just explained: the Board itself found that the safety and efficacy of reducing or arresting glycogen buildup in muscle could only be determined through human clinical trials, which had not yet been conducted. There is no basis

in the record to conclude that a POSA would have reasonably expected to safely and effectively achieve the results claimed, particularly given BioMarin's expert's concession that there was **no** known correlation between GAA dose or dosing schedule and the reduction or arrest of further glycogen buildup. *Leo Pharm. Prods. Ltd.*, 726 F.3d at 1357.

In any event, the Board simply asserted the conclusion that its ultimate findings would "remain the same" without offering any "supporting reasons for [its] conclusions," as the APA unambiguously requires. 5 U.S.C. § 557(c)(3). This is particularly problematic because Board recognized that targeting muscle was more "controversial," and thus the evidence suggested there was a lesser "expectation of success with regard to" that tissue. A46; A13. And indeed, the Board in its decisions rejected Genzyme's argument that a POSA "would not have had a reasonable expectation of successfully reducing or arresting further accumulation of glycogen in a patient's muscle cells" precisely **because** Genzyme's arguments were "premised on a claim construction that would require the phrase 'glycogen in the patient' [in the challenged claims] necessarily to include lysosomal glycogen in skeletal muscle," a claim construction the Board did not adopt. A41; A14-15. The unexplained change in course between this conclusion and its unreasoned statement that its findings would "remain the same" regardless of the claim construction must also be set aside as arbitrary and

capricious. *See* 5 U.S.C. § 706(2)(A); *General Chem. Corp. v. United States*, 817 F.2d 844, 846 (D.C. Cir. 1987) (finding the agency action was arbitrary and capricious because its analysis was "internally inconsistent and inadequately explained").

Under a proper claim construction, judgment must be entered in favor of Genzyme.

IV. THE BOARD'S OBVIOUSNESS DETERMINATION MUST BE SET ASIDE

Independent of the proper claim construction, the Board's determination that the challenged claims of the '410 and '226 patents are invalid as obvious must be set aside. The Board failed to determine the level of ordinary skill in the art as required under *Graham*. Such an error generally results in remand, but there is no need for remand here because BioMarin submitted no relevant evidence in the record that could support the conclusion that a POSA would have had a reasonable expectation of obtaining the results set forth in the whereby clauses of the claims. *See* 5 U.S.C. § 706(2)(E) (agency decisions unsupported by substantial evidence must be set aside). The Board's obviousness determination was instead impermissibly premised on the Board's own independent, subjective analysis of the prior art, and must be set aside for this reason as well.

A. The Board Failed To Determine The Level Of Ordinary Skill In The Art As Required By *Graham*

It is well established that, "**before** invalidating a patent for obviousness," several factual findings—known as "*Graham* findings"—must be made. *Ruiz v. A.B. Chance Co.*, 234 F.3d 654, 663 (Fed. Cir. 2000) (emphasis added) (citing *Graham*, 383 U.S. at 17). In particular, "to determine obviousness as a legal matter, four factual inquiries must be made concerning: 1) the scope and content of the prior art; 2) the level of ordinary skill in the art; 3) the differences between the claimed invention and the prior art; and 4) secondary considerations of nonobviousness." *Ruiz*, 234 F.3d at 662.

As this Court has explained, "the need for express *Graham* findings takes on an especially significant role because of an occasional tendency of district courts to depart from the *Graham* test, and from the statutory standard of unobviousness that it helps determine, to the tempting but forbidden zone of hindsight." *Id.* at 663 (quoting *Loctite Corp. v. Ultraseal Ltd.*, 781 F.2d 861 (Fed. Cir. 1985)). Thus, while a district court—or, in this case, the Board—need not necessarily mention *Graham*, its decision must demonstrate that "the required factual determinations were actually made" and it must be "clear that they were considered while applying the proper legal standard of obviousness." *Id.* at 664 (quoting *Specialty Composites v. Cabot Corp.*, 845 F.2d 981, 990 (Fed. Cir. 1988)).

Here, the Board failed to make a specific factual finding regarding the level

of ordinary skill in the art—a required component of the obviousness analysis under *Graham*. That suffices to require reversal here. Indeed, the "importance of resolving the level of ordinary skill in the art lies in the **necessity of maintaining objectivity** in the obviousness inquiry," *Ryko Mfg. Co. v. Nu-Star, Inc.*, 950 F.2d 714, 718 (Fed. Cir. 1991) (emphasis added), and—as explained below—the absence of such a determination resulted in the Board's obviousness analysis being impermissibly subjective and dependent on its independent expertise.

B. The Record Evidence Does Not Support An Obviousness Finding From The Perspective Of A POSA At The Time Of The Invention

In any event, the Board's obviousness conclusion suffers from a fundamental problem—the record lacks sufficient evidence to support an obviousness finding from the perspective of a POSA at the time of the invention.

It is well-established that the obviousness analysis is an **objective analysis**. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406, 421 (2007). The "relevant inquiry" under this objective standard "is what a **hypothetical ordinarily skilled artisan [i.e., a POSA]** would have gleaned from the cited references at the time that the patent application . . . was filed." *Amazon.com, Inc. v. Barnesandnoble.com, Inc.*, 239 F.3d 1343, 1364 (Fed. Cir. 2001) (emphasis added). And the Board may **not**, in a contested proceeding such as an IPR, use its own specialized expertise to fill in evidentiary gaps to support its ultimate conclusions. Rather, the "Board's role in such cases [is] as an impartial

adjudicator of an adversarial dispute between two parties," so "**it is impermissible for the Board to base its factual findings on its expertise**, rather than on evidence in the record." *Brand*, 487 F.3d at 869 (emphasis added); *see also* 5 U.S.C. §§ 556(e), 557(a)-(b) (agency determinations in formal adjudications must be made based only on evidence of record). Indeed, the requirement that the Board's decision be grounded in the record is particularly important in the context of an IPR proceeding, because Congress has expressly placed the burden of proof on the petitioner. 35 U.S.C. § 316(e). Allowing the Board to rely on its own independent expertise rather than evidence presented by the petitioner would eviscerate the statutory requirement that the petitioner prove its case.

Here, the Board's obviousness determination must be set aside because BioMarin did not submit **objective** evidence to support the conclusion that a **POSA** would have had a reasonable expectation of obtaining the results set forth in the whereby clauses of the claims. The Board's contrary conclusion impermissibly rests on its own expertise.

1. *The Record Lacks Substantial Evidence From Which The Board Could Conclude That BioMarin Met Its Burden Of Proving That A POSA Would Have Had A Reasonable Expectation Of Achieving The Results Claimed In The Patents At The Time Of The Invention*

BioMarin submitted little to no evidence to establish a POSA's perspective at the time of the invention. Indeed, although BioMarin's expert Dr. Pastores nominally makes reference to a person "knowledgeable and skilled" in the field,

A1212-13, his direct testimony is devoid of any definition of the level of skill possessed by a hypothetical POSA, and there is no evidence that his direct testimony was conducted from the **objective** perspective of such a person. In fact, Dr. Pastores conceded that he conducted a purely **subjective and hindsight-infected** analysis.

Dr. Pastores repeatedly characterized his opinions in terms of **his** perspective and what **he** understood based on the prior art, rather than what a **POSA** would have known or understood. And he admitted on cross-examination that he did not consider the art from the perspective of any individual or group of individuals other than himself. *See* A3240 ("Q. Well, you provided your opinion, correct? A. Yes. **Q. From your perspective, correct? A. Yes.** Q. So you did **not** examine the information that was available through the '90s from the perspective of any other particular individual or group of people, correct? A. Yes."); A3240-41 ("When I was asked to review the state of the art in the early to mid-1990s, I was asked to review of what I understood was available in the general medical literature. And I looked at it also within the context of what **I** would have understood then the body of literature was telling **me** based on **my** knowledge and experience at that time."); *see also, e.g.,* A1208 ("**My** sense from reading the article . . ."); A1221 ("Thus, in my opinion the three criteria that **I** would have considered in evaluating whether ERT using a CHO produced recombinant GAA

protein would likely be successful. . . . From the information that was available at the time (as of December 6, 1997) ***I* would have had a high expectation of seeing an efficacy in treating** Pompe with recombinant GAA produced in CHO cells."); A1225 (". . . **I** would have expected . . ."); A1230 (". . . **I** would expect . . ."); A1231-32 (". . . **I** would have fully expected. . . " and " . . . **I** would fully expect . . .") (all emphases added).

Accordingly, Dr. Pastores' testimony fails to provide any **objective** factual evidence or support for a determination of what the prior art would have taught a POSA, in violation of the Supreme Court's mandate under *KSR*. *See* 550 U.S. at 406, 421. Whatever Dr. Pastores "did or did not **personally** realize at the time based on his actual knowledge is irrelevant," since the "relevant inquiry is what a hypothetical ordinarily skilled artisan would have gleaned from the cited references" at the time of the invention. *Amazon.com*, 239 F.3d at 1364 (emphasis in original). Nor does "testimony as to how experts could have made the patented invention . . . show that such manipulation would have been obvious to one of ordinary skill." *Rotron Inc. v. ITC*, 845 F.2d 1034 (Fed. Cir. 1988). Simply put, Dr. Pastores' personal assessment of the prior art is irrelevant to the analysis. But his personal perspective is all that Dr. Pastores provided here.

Dr. Pastores also **admitted** to the impermissible use of hindsight. He conceded on cross-examination that he had **applied his present-day knowledge** in

assessing the prior art, and further acknowledged that he did not "know how one would separate your current body of knowledge from what your knowledge was way back in time." A3240-41. Yet the very thing Dr. Pastores admitted he did not know how to do is exactly what this Court's precedent requires. Again, there must be evidence of what a "hypothetical ordinarily skilled artisan would have gleaned from the cited references at the time that the patent application . . . was filed." *Amazon.com*, 239 F.3d at 1364. The Board's finding of obviousness must be reversed because there is no record evidence to support such a finding.

2. *The Absence Of Record Evidence Supporting A Reasonable Expectation Of Success Finding From The Perspective Of A POSA Cannot Be Cured By The Board*

As explained above, the Board cannot cure, through the application of its own expertise, BioMarin's failure to submit evidence sufficient to establish that a **hypothetical POSA at the time of the invention and without use of hindsight** would have had a reasonable expectation of successfully arresting or reducing the accumulation of glycogen—i.e., the Board cannot cure BioMarin's failure to meet its statutory burden of proof under 35 U.S.C. § 316(e). Yet that is exactly what happened here. The Board in finding the challenged claims obvious could only have itself supplied the absent, but necessary, evidentiary support for its reasonable-expectation-of-success analysis.

This is impermissible. Again, in contested matters, "**the Board cannot**

simply reach conclusions based on its own understanding or experience—or on its assessment of what would be basic knowledge or common sense." *In re Zurko*, 258 F.3d 1379, 1385-86 (Fed. Cir. 2001) (emphasis added). "Rather, the Board must point to some concrete evidence in the record in support of these findings. To hold otherwise would render the process of appellate review for substantial evidence on the record a meaningless exercise." *Id.* at 1386; *see Brand*, 487 F.3d at 868. In fact, the Board has itself previously acknowledged that it is not permitted to fill in gaps in the evidence relating to the expectations of a POSA. *See Robertson v. Timmermans*, 2008 WL 6484546, at *8 (B.P.A.I. Nov. 21, 2008) ("We are also **prohibited** from making a determination [relating to the motivation to combine] **based on our own knowledge and expertise. The Board cannot substitute its own expertise for evidence that is not in the record**." (emphasis added) (citing *Brand*, 487 F.3d at 869)).

The Board's use of its own expertise to support its obviousness finding means that its final decisions cannot stand. And because BioMarin failed to submit evidence from which the Board could have made the necessary reasonable-expectation-of-success finding from the perspective of a POSA during the relevant period, BioMarin failed to carry its burden of proof. The Board should have entered judgment for Genzyme.

CONCLUSION

For the foregoing reasons, the final decisions of the Board should be reversed and BioMarin's petitions finally rejected.

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ADDENDUM

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Paper 81
Entered: February 23, 2015

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BIOMARIN PHARMACEUTICAL INC.,
Petitioner,

v.

GENZYME THERAPEUTIC PRODUCTS LIMITED PARTNERSHIP,
Patent Owner.

Case IPR2013-00534
Patent 7,351,410

Before LORA M. GREEN, JACQUELINE WRIGHT BONILLA, and SHERIDAN K. SNEDDEN, *Administrative Patent Judges.*

SNEDDEN, *Administrative Patent Judge*.

FINAL WRITTEN DECISION
35 U.S.C. § 318(a) and 37 C.F.R. § 42.73

I. INTRODUCTION

BioMarin Pharmaceutical Inc. ("Petitioner") filed a petition requesting *inter partes* review of claim 1 (Paper 1, "Pet.") of Patent No. 7,351,410 B2 (Ex. 1001; "the '410 patent"). We instituted trial for the challenged claims on the following grounds of unpatentability asserted by Petitioner:

Reference(s)	Basis	Claims challenged
Reuser,[1] Barton,[2] and Van der Ploeg[3]	§ 103	1
Duke Press Release,[4] Barton, and Van der Ploeg	§ 103	1

Decision to Institute (Paper 9, "Dec.") 9.

After institution, the Board of Trustees of Genzyme Therapeutic Products Limited Partnership ("Patent Owner"), filed its Patent Owner's Response ("Resp."). Paper 41. Petitioner filed a Reply (Paper 51, "Reply"). Patent Owner did not file a motion to amend claims.

Petitioner relies upon the declarations of Dr. Gregory M. Pastores ("Pastores Declaration") (Ex. 1030) and Dr. Matthew Croughan ("Croughan Declaration") (Ex. 1033) in support of its Petition. Patent Owner relies upon the declaration of William Canfield, M.D., Ph.D. ("Canfield Declaration")

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[1] Reuser et al., WO 97/05771, published Feb. 20, 1997 (Ex. 1005).

[2] Barton et al., *Replacement Therapy for Inherited Enzyme Deficiency – Macrophage-Targeted Glucocerebrosidase for Gaucher's Disease*, 324 *N. ENG. J. MED.* 1464-1470 (1991) (Ex. 1004).

[3] Van der Ploeg et al., *Receptor-Mediated Uptake of Acid α-Glucosidase Corrects Lysosomal Glycogen Storage in Cultured Skeletal Muscle*, 24(1) *PEDIATRIC RESEARCH* 90-94 (1988) (Ex. 1032).

[4] Duke University, *Duke Obtains FDA Designation for Pompe Disease Therapy*, press release dated September 2, 1997, 2 pages (Ex. 1002).

(Ex. 2041) in support of its Response.

Petitioner filed a Motion to Exclude certain of Patent Owner's evidence. Paper 55. Patent Owner filed an Opposition (Paper 63), and Petitioner filed a Reply (Paper 65).

Patent Owner filed a Motion to Exclude certain of Petitioner's evidence. Paper 57. Petitioner filed an Opposition (Paper 60), and Patent Owner filed a Reply (Paper 66).

Oral argument was conducted on October 3, 2014. A transcript is entered as Paper 79 ("Tr.").

This Final Written Decision addresses challenges to the patentability of claim 1. Petitioner has shown by a preponderance of the evidence that claim 1 of the '410 patent is unpatentable.

A. Related Matters

The Parties represent that there are no related matters. Pet. 1; Paper 8; Paper 35.

On the same day Petitioner filed its petition in this proceeding, however, it also filed two other Petitions seeking *inter partes* review of U.S. Patent No. 7,056,712 (IPR2013-00535) and U.S. Patent No. 7,655,226 (IPR2013-00537), both of which are related to methods of treating Pompe disease.

B. The '410 patent (Ex. 1001)

The technology of the patent is enzyme-replacement therapy for patients with Pompe disease, which is caused by deficiency of the lysosomal enzyme acid α-glucosidase ("GAA"). Ex. 1001, 1:59–61. The deficiency in the lysosomal enzyme results in harmful accumulation of glycogen in

muscle. *Id.* at 15:26–29. The patent discloses a method for treating Pompe disease comprising administering to the patient a therapeutically effective amount of human GAA. *Id.* at 2:34–36. Doses of human GAA may be administered two weeks apart. *Id.* at 24:22–23.

C. The Claim

Claim 1 is the only claim of the '410 patent, and is reproduced below:

> 1. A method of treating a human patient with Pompe's disease, comprising intravenously administering biweekly to the patient a therapeutically effective amount of human acid alpha glucosidase, whereby the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested.

II. DISCUSSION

A. Claim Interpretation

In an *inter partes* review, claim terms in an unexpired patent are interpreted according to their broadest reasonable construction in light of the specification of the patent in which they appear. 37 C.F.R. § 42.100(b); Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,766 (Aug. 14, 2012). Claim terms are given their ordinary and customary meaning, as would be understood by one of ordinary skill in the art in the context of the entire disclosure. *In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007). Any special definition for a claim term must be set forth in the specification with reasonable clarity, deliberateness, and precision. *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

We expressly interpret below only those claim terms that require analysis to resolve arguments related to the patentability of the challenged claims.

1. Construction of the phrase "whereby the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested"

We construe claim 1 to be directed to a method of treating a human patient with Pompe disease. The claimed method comprises a single step: "intravenously administering biweekly to the patient a therapeutically effective amount of human acid alpha glucosidase." Claim 1 further recites the result achieved from the practice of the method recited in claim 1. Specifically, the step of intravenously administering biweekly to the patient a therapeutically effective amount of human GAA results in the reduction in the concentration of accumulated glycogen in the patient and/or the arrest of further accumulation of glycogen. Thus, the recited whereby clause defines what is achieved from the administration of "a therapeutically effective amount of human acid alpha glucosidase" to a human patient with Pompe disease.

As to what is required in order to achieve the result defined by the whereby clause, Patent Owner proposes a construction that would necessarily include "lysosomal glycogen in the skeletal muscle" within the meaning of the phrase "glycogen in a patient." Resp. 12–16. Patent Owner argues that such a construction is necessary because skeletal muscle must be targeted in order to effectively treat Pompe disease. *Id.* (citing Ex 1162, 62:5-22). Patent Owner further directs our attention to a section of the '410 patent that describes complications and treatment for both the infantile and adult forms of Pompe disease. *Id.* (citing Ex 1001, 15:12–58). For the infantile form, "lysosomal glycogen storage is observed in various tissues, and is most pronounced in heart and skeletal muscle." Ex. 1001, 15:26–28. For the adult form, "skeletal muscle weakness is the major problem;

cardiomegaly, hepatomegaly, and macroglossia can be seen, but are rare." *Id.* at 15:48–50.

Petitioner notes that the whereby clause of claim 1 was added during the prosecution of the '410 patent in a paper entered June 1, 2006. Reply 1–2 (citing Ex. 1022, 3). That paper directs the reader to where the written description support for the addition of the whereby clause may be found in the specification of the application that issued as the'410 patent. Ex. 1022, 3. The passage of the specification cited by the inventor during prosecution for support of the amendment provides as follows:

> When two KO mice were injected 4 times every 6 days (experiment B), a marked decrease of total cellular glycogen was observed in both heart and liver. No effects were observed in skeletal muscle tissues with regard to total glycogen. . . .
>
> The results showed that mice treated 13 weeks with 0.5 mg / mouse (Group A, 3 animals/Group) had an increase of activity in the liver and spleen and decreased levels of glycogen in liver and perhaps in heart. One animal showed increased activity in muscles, although there was no significant decrease of glycogen in muscle.

Ex. 1062, 35:1–3, 31–34. That passage of the Specification of the '410 patent summarizes an *in vivo* experiment that resulted in "a marked decrease of total cellular glycogen was observed in both heart and liver," but not in skeletal muscle tissue.

After consideration of the arguments presented by the parties, we do not find that the record supports Patent Owner's construction that would necessarily include "lysosomal glycogen in the skeletal muscle" within the meaning of the phrase "glycogen in a patient." We note that the claim does not recite specific organs or tissue, does not recite any specific form of

Pompe disease, and does not require, for example, the patient to experience an increased life-span. The whereby clause merely requires the reduction or arrest of glycogen in the patient. In that regard, the evidence highlighted by Petitioner supports a conclusion that the whereby clause is reasonably interpreted to encompass the scenario where the reduction or arrest of glycogen is observed solely in the heart or liver. Accordingly, we conclude that the broadest reasonable interpretation of the phrase "glycogen in a patient" does not necessarily include "lysosomal glycogen in the skeletal muscle." Rather, the broadest reasonable interpretation of the phrase "glycogen in a patient," as would be understood by the ordinary artisan in light of the Specification, encompasses lysosomal glycogen in the skeletal muscle, heart, or liver.

2. Construction of the term "biweekly"

Patent Owner construes the term "biweekly" to mean "at least two or more intravenous administrations with each administration separated by two weeks." Resp. 16. Petitioner agrees. *See* Pet. 29 (defining "biweekly" to mean "that the drug or composition is administered once, followed by a second administration two weeks later (as opposed to meaning twice in one week").

However, the parties disagree as to whether the element of "biweekly" is met by a dosing regimen lasting only two weeks—that is, a single administration, followed only by a single second administration two weeks later. Resp. 16; Pet. 29–30. While Petitioner correctly points out that the claim does not require a prolonged period of administration, the claim does require the treatment of Pompe disease, which is effected via the reduction or arrest of accumulated glycogen. As such, we construe the claim to

require biweekly administration for a period of time sufficient to achieve the result required by the claim.

B. The Prior Art

1. Summary of Reuser (Ex. 1005)

Reuser discloses the production of phosphorylated lysosomal proteins using transgenic nonhuman mammals for use in enzyme replacement therapy as treatment for lysosomal enzyme deficiencies. Ex. 1005, Abstract, 18:12–14. Reuser expressly discloses Pompe disease[5] as such a lysosomal enzyme deficiency. *Id.* at 2:13–29. With regard to the treatment of Pompe disease, Reuser discloses as follows:

> For lysosomal diseases other than Gaucher disease the evidence suggests that enzyme therapy is most effective when the enzyme being administered is phosphorylated at the 6' position of a mannose side chain group. *For [Pompe disease this has been tested by intravenously administering purified acid α-glucosidase in phosphorylated and unphosphorylated forms to mice and analyzing uptake in muscle tissue.* The highest uptake was obtained when mannose 6-phosphate-containing enzyme was used (Van der Ploeg et al., Pediat. Res. 28, 344-347 (1990); J. Clin. Invest. 87, 513-518 (1991)).[6]

Id. at 2:35–3:10 (emphasis added).

Reuser expressly identifies GAA as an enzyme useful for production in the disclosed transgenic animal systems. *Id.* at 4:36–37.[7] Specific to human GAA, Reuser discloses a map of several transgenes containing GAA

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[5] Also referred to as Glycogen Storage Disease Type II (GSD II).

[6] Citations provided by Petitioner as Ex. 1051 and Ex. 1009, respectively.

[7] Reuser also discloses the use of a stable eukaryotic cell line transfected with the acid α-glucosidase gene for the purposes of producing the human acid α-glucosidase protein. *Id.* at 3:15–18.

cDNA[8] or genomic DNA[9] and details experiments in which a mannose 6-phosphate containing human GAA was produced in the milk of transgenic mice. *Id.* at 21:14–28:24.

Reuser contemplates pharmaceutical compositions for use in enzyme replacement therapeutic procedures, and specifically pharmaceutical compositions for intravenous administration. *Id.* at 18:36–20:28. Furthermore, Reuser provides general guidance with regard to dosage and dosage regimen. Specifically, Reuser provides as follows:

> For individuals at risk of lysosomal enzyme deficiency disease, the pharmaceutical composition [sic] are administered prophylactically in an amount sufficient to either prevent or inhibit accumulation of metabolite. An amount adequate to accomplish this is defined as a "therapeutically-" or "prophylactically-effective dose." Such effective dosages will depend on the severity of the condition and on the general state of the patient's health, but will generally range from about 0.1 to 10 mg of purified enzyme per kilogram of body weight.

Id. In the case of Pompe disease, glycogen is the metabolite. *Id.* at 2:13–29.

2. Summary of Barton (Ex. 1004)

Barton describes a clinical trial in which patients with Gaucher Disease[10] were administered glucocerebrosidase for enzyme replacement therapy on a biweekly intravenous administration schedule. Ex. 1004, Abstract.

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[8] Ex. 1005, Fig. 1.

[9] Ex. 1005, Fig. 2.

[10] Gaucher disease is a lysosomal storage disorder caused by an insufficiency of glucocerebrosidase. Ex. 1004, 1464.

3. Summary of Van der Ploeg (Ex. 1032)

Van der Ploeg describes an *in vitro* study using cultured skeletal muscle cells from a patient with Pompe disease. Ex 1032, Abstract. Cultured skeletal muscle cells were incubated with GAA containing mannose-6-phosphate purified from human urine. *Id.* Van der Ploeg reports that the "[e]fficient uptake of acid α-glucosidase was achieved by using the mannose-phosphate receptor on the cell surface as a target for an enzyme precursor with phosphorylated high-mannose types carbohydrate chains purified from human urine." *Id.* The enzyme was reported to have a half-life of 6–9 days. *Id.* at 91.

4. Summary of Duke Press Release (Ex. 1002)

The Duke Press Release reports the U.S. Food and Drug Administration ("FDA") approval of Duke University's application for Orphan Drug Designation for a new therapy for Pompe disease. Ex. 1002; Ex. 1182. The Duke Press Release describes Pompe disease as an inherited deficiency of the enzyme acid alpha glucosidase and provides a discussion of how the therapy, developed at Duke University, "will be tested in infants with the most severe symptoms and for whom the disease is fatal." *Id.* With regard to how the therapy would be tested, the Duke Press Release provides as follows:

> The Duke clinical trial will test a genetically engineered form of the enzyme, expressed in a cell line developed in the laboratory of Dr. Y. T. Chen, chief of the Division of Medical Genetics in the department of pediatrics. Initially, the drug will be tested in a small number of Pompe disease infants to evaluate the safety and efficacy of the recombinant enzyme treatment. . . .

> Chen, who will lead the clinical trial, anticipates that recombinant enzyme injected into infants will be taken up by their muscle cells and restore normal glycogen levels. This treatment, known as an enzyme replacement therapy, would be required for the rest of these patients' lives.
>
> He said he hopes to expand the treatment to additional Pompe disease patients as safety and efficacy are demonstrated and supplies of the enzyme are available. Chen's team at Duke has spent more than five years developing the cell line that produces the recombinant drug.

Id.

C. Obviousness of Claim 1

1. Obviousness of Claim 1 over the Combination of Reuser, Barton, and Van der Ploeg

Petitioner has presented evidence showing that Reuser, Barton, and Van der Ploeg disclose every limitation of claim 1. Pet. 40–45. Reuser discloses intravenous pharmaceutical compositions containing human GAA for use in enzyme replacement therapy for the treatment of Pompe disease. *Id.*; Ex. 1005 (*see* Section II.B.1 above). The only limitation in claim 1 not expressly disclosed in Reuser is the "biweekly" limitation. *Id.* at 44–45. For this element of claim 1, Petitioner combines Reuser with Barton and Van der Ploeg, and argues that biweekly administration would have been the preferred dosing schedule based on experience with Gaucher disease (*id.* (citing Ex. 1004)) and based on the half-life of GAA (*id.* (citing Ex. 1032)). Petitioner argues further that "determination of how much and how often to administer the enzyme to the particular patient is a matter of routine optimization" (*id.* at 5) and that "[t]he inventors of the '410 patent simply followed a typical drug development pathway that was laid out in the prior

art, where the use of GAA to successfully treat Pompe disease in a human patient was a predictable outcome based on previous *in vitro* and *in vivo* studies" (*id.* at 8).

Patent Owner does not contend that the combination of references fails to address each element of claim 1. Rather, Patent Owner contends that a person of ordinary skill in the art would not have had a reasonable expectation of success for a method of treating a human patient with Pompe disease using GAA administered biweekly to reduce and/or arrest further accumulation of glycogen in the skeletal muscle of a human patient. Resp. 40–45, 47–49. Patent Owner contends further that a person of ordinary skill in the art would not have had motivation to combine Barton with Reuser and van der Ploeg due to the significant differences between Gaucher disease and Pompe disease. *Id.* at 45–47. Patent Owner additionally argues the existence of objective indicia of non-obviousness, including long-felt need, skepticism, industry praise, and commercial success. *Id.* at 56–59.

We begin our analysis by addressing the key question of whether the biweekly administration of GAA to a patient with Pompe disease was nothing more than the result of routine optimization that would have been obvious to one of ordinary skill in the art. The record does not contain any evidence that human clinical trials were initiated prior to December 7, 1998, the priority date of the '410 patent. Accordingly, a person of ordinary skill in the art could not have predicted with absolute certainty that a safe and effective dosing regimen for using GAA in a method of treating Pompe disease could be achieved.[11] For example, a skilled artisan would have

[11] We recognize that "absolute predictability of success" is not the criterion; "[f]or obviousness under § 103, all that is required is a reasonable

understood that to treat Pompe disease effectively using GAA, sufficient quantities of enzyme would have to reach the patient's muscle cells, which could potentially require high doses that could introduce safety and efficacy hurdles resolvable only with human clinical trials.[12] Resp. 41–42 (citing Ex 2041 ¶ 105; Ex 2042 ¶¶ 67-68, 86, 99, 121-122; Ex 1162, 63:14-64:13, 67:2-11; Ex. 1030 ¶ 75; Ex 1011).

Despite this recognized difficulty, however, we are persuaded that, under the facts of this case, a person of ordinary skill would have been motived to pursue the clinical development of the therapy disclosed in Reuser. *See* Section II.B.1 for summary of Rueser. Given that Reuser discloses a pharmaceutical composition containing GAA, a method of making the composition using transgenic animals, and a method of using the composition in an enzyme replacement therapy for the treatment of Pompe disease, what remained to be achieved to arrive at the subject matter of claim 1 was a biweekly dosing schedule for the disclosed therapeutic. A

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expectation of success." *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988).

[12] As discussed in Section II.A.1, we do not interpret the claim to necessarily require the reduction or arrest of stored glycogen in skeletal muscle, but understand that achieving such a result is important for the complete treatment of both forms of Pompe disease. Nonetheless, we include the concept of reduction or arrest of stored glycogen in skeletal muscle in our analysis as we do not find its inclusion to hinder our analysis. That is, whether we include or exclude the result of reduction or arrest of stored glycogen in skeletal muscle within the scope of claim 1, our conclusions remain the same, albeit achievement of the reduction of glycogen in the heart and liver appear to be less controversial, and thus the evidence suggests there was a greater expectation of success with regard to these tissues.

preponderance of the evidence establishes that the selection of the dose and dosing schedule would have been a routine optimization of the therapy outlined in Reuser (Ex. 1005, 18:36–20:28), which would have been achievable through the use of standard clinical trial procedures (Ex. 1030 ¶¶ 74–90).

We conclude that the experimentation needed to achieve biweekly administration recited in claim 1 was "'nothing more than the routine' application of a well-known problem-solving strategy, . . . 'the work of a skilled [artisan], not of an inventor.'" *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1368 (Fed. Cir. 2007) (quoting *Merck & Co. v. Biocraft Labs., Inc.*, 874 F.2d 804, 809 (Fed. Cir. 1989); *DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1371 (Fed. Cir. 2006)); *see also In re Aller*, 220 F.2d 454, 456 (CCPA 1955) ("[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation."); *In re Boesch*, 617 F.2d 272, 276 (CCPA 1980) ("[D]iscovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art."). The motivation to optimize the therapy disclosed in Reuser "flows from the 'normal desire of scientists or artisans to improve upon what is already generally known.'" *Pfizer,* 480 F.3d at 1348 (quoting *In re Peterson*, 315 F.3d 1325, 1330 (Fed. Cir. 2003)).

Patent Owner contends that a person of ordinary skill in the art would not have had a reasonable expectation of successfully reducing or arresting further accumulation of glycogen in a patient's muscle cells. Resp. 47–49. That argument is premised, however, on a claim construction that would require the phrase "glycogen in the patient" in claim 1 necessarily to include

lysosomal glycogen in skeletal muscle. *Id.* at 15. As discussed above in Section II.A.1, we do not adopt Patent Owner's proposed claim construction in this regard. As such, we do not understand Patent Owner's arguments with regard to reasonable expectation of success to be applicable to the question of whether or not a person of ordinary skill in the art would have had a reasonable expectation of successfully reducing or arresting further accumulation of glycogen in a patient's other tissues such as heart and liver.

To the extent that Patent Owner's arguments are applicable under our claim construction, we note that a reasonable expectation of success does not require absolute predictability. *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). The fact that a suggested dose (as taught in Reuser) and dosing schedule had not been established yet as safe and effective in human clinical trials at the time of invention does not demand a conclusion of nonobviousness.

While we recognize that there would have been some degree of unpredictability for the successful treatment of Pompe disease from the administration of GAA, the preponderance of evidence of record suggests all that remained to be achieved over the prior art was the determination that a specific dose within a previously suggested dose range, and its corresponding dosing schedule, would have been safe and effective for the treatment of human patients. That is, this is not a case where the prior art teaches merely to pursue a "general approach that seemed to be a promising field of experimentation" or "gave only general guidance as to the particular form of the claimed invention or how to achieve it." *O'Farrell*, 853 F.2d at 903; *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1167 (Fed. Cir. 2006).

By December 7, 1998, the field related to the development of an enzyme replacement therapy for the treatment of Pompe disease had developed to the point at which 1) it was recognized that GAA must be post-translationally modified with mannose-6-phosphate to promote cellular uptake through a mannose-6-phosphate receptor *in vitro*;[13] 2) *in vivo* studies had been performed in which GAA containing mannose-6-phosphate was intravenously administered to mice[14] and Japanese Quail;[15] 3) it was known that mannose 6-phosphate containing human GAA could be produced in the milk of transgenic animals;[16] and 4) the FDA was granting applications for orphan drug designation for enzyme replacement therapy for Pompe disease using recombinant GAA.[17]

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[13] Ex. 1005 at 2:35–3:10.

[14] Ex. 1009 presents data suggesting that GAA containing mannose 6-phosphate is taken up in the skeletal muscle and heart of mice after intravenous administration.

[15] Ex. 1007 presents data suggesting that the intravenous administration of human GAA to GAA-deficient Japanese reduced glycogen levels in the heart, liver and muscle and produced muscle improvement. Ex. 1007, abstract. The authors conclude that "[t]hese data also suggest enzyme replacement with recombinant human GAA is a promising therapy for human Pompe disease." *Id.*

[16] Ex. 1005 at 21:14–28:24.

[17] Ex. 1002; Ex. 1182. We further note that the FDA application process requires an applicant to provide "enough information to establish a medically plausible basis for expecting the drug to be effective in the rare disease." Ex. 1029. Furthermore, as stated by Dr. Canfield, "a [skilled artisan] would know, and would understand from Ex 1002, that the purpose of the proposed clinical trial would be to evaluate whether the administered enzyme was safe and effective in humans and to determine the appropriate dose." Ex 2041 ¶ 81 (citing Ex 1002, 2).

Furthermore, this is not a case where there were "numerous parameters" to try. *Pfizer*, 480 F.3d at 1364 (citing *Medichem*, 437 F.3d at 1165 ("to have a reasonable expectation of success, one must be motivated to do more than merely to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.") (internal quotations omitted)). Rather, we are persuaded by Dr. Pastores's testimony that the knowledge in the art regarding the treatment of Pompe disease with human GAA would have provided the motivation to select a suitable dose and dosing schedule (Ex. 1030 ¶ 38), would have been informed by the clinical experience with Gaucher disease (*id.* at ¶ 74 (citing Ex. 1004, 1056, 1057)), and that, because "it was well known that any enzyme replacement therapy for Pompe disease would be required for the rest of a patient's life, . . . repeated spaced administration of GAA to patients would be immediately understood upon reading [Reuser]" (*id.* at ¶ 58).

In view of the above, we conclude that a person of ordinary skill in the art would have had a reasonable expectation of success at the time the invention was made. What remained was the execution of human clinical trials, arguably "routine" to a person of ordinary skill in the art, to verify the expectation that a specific dosage (within a previously suggested dosage range) and corresponding dosage regimen would have been safe and effective. *Cf. Pfizer*, 480 F.3d at 1367 ("[E]xperiments used by Pfizer's scientists to verify the physicochemical characteristics of each salt are not equivalent to the trial and error procedures often employed to discover a new compound where the prior art gave no motivation or suggestion to make the

new compound nor a reasonable expectation of success."); *Velander v. Garner*, 348 F.3d 1359, 1368 (Fed. Cir. 2003) (stating that one skilled in the art would view variability in producing fibrinogen in transgenic mammals as evidence that "expense, time and effort" would be involved did not equate to a conclusion that success was unlikely).

Finally, we note that the absence in the record of evidence identifying a difference between the prior art and the subject matter of the claims further persuades us that no more than routine processes were needed to achieve the results recited in claim 1. For example, the absence of any discussion with regard to the unexpected or superior results associated with the biweekly administration feature of claim 1 further persuades us that the subject matter of claim 1 was a product of routine clinical trial processes. As such, we conclude that the prior art brought the subject matter of claim 1 within the technical grasp of a person of ordinary skill in the art rendering it obvious, absent objective evidence of nonobviousness. *Pfizer*, 480 F.3d at 1344 ("[O]bviousness cannot be avoided simply by a showing of some degree of unpredictability in the art so long as there was a reasonable probability of success.").

2. *Obviousness of Claim 1 over the Combination of Duke Press Release, Barton, and Van der Ploeg*

Petitioner contends that claim 1 is unpatentable under 35 U.S.C. § 103(a) as being obvious over the combination of Duke Press Release (Ex. 1002), Barton (Ex. 1004), and van der Ploeg (Ex. 1032). For the reasons expressed below, we conclude that Petitioner has demonstrated by a preponderance of evidence that claim 1 would have been obvious over those references.

The Duke Press Release details a proposed FDA clinical trial study in which infants with Pompe disease would be injected with recombinant GAA to evaluate the safety and efficacy of the recombinant enzyme treatment. Ex. 1002; Pet. 31-32. Patent Owner contends that the Duke Press Release does not disclose the dosing regimen used for the planned study, and thus does not expressly disclose the feature of intravenously administering human enzyme biweekly as recited in claim 1. Resp. 30 (citing Ex 2041 ¶¶ 80–83). We are persuaded by Petitioner's argument, however, that the disclosure in the Duke Press Release of "injected" is a reference to intravenous administration. Pet. 32 (citing Ex. 1030 ¶ 38). Furthermore, additional evidence of record supports a conclusion that intravenous administration would have been a preferred route of administration for enzyme replacement therapy. Ex. 1004 (disclosing biweekly intravenous administration of glucocerebrosidase to patients with Gaucher Disease); Ex. 1005, claim 19 (disclosing a pharmaceutical composition containing human GAA and a pharmaceutical carrier for intravenous administration).

The Duke Press Release does not disclose the source of enzyme to be used in the trial, and thus does not disclose the use of human GAA. Resp. 30 (citing Ex. 2041 ¶ 83). That deficiency, however, is cured by Van der Ploeg, which describes an *in vitro* study using cultured skeletal muscle cells from a patient with Pompe disease and human GAA. Ex 1032, Abstract. Van der Ploeg reports that the "[e]fficient uptake of acid α-glucosidase was achieved by using the mannose-phosphate receptor on the cell surface as a target for an enzyme precursor with phosphorylated high-mannose types carbohydrate chains purified from human urine." *Id.* Thus, the concept of using of human GAA to treat Pompe disease was known in the art.

Patent Owner makes several arguments related to the dose and dosing schedule elements of claim 1. First, Patent Owner argues that the Duke Press Release contains no dosing information, and, therefore, does not contain a disclosure that satisfies the "therapeutically effective amount" element of claim 1. Resp. 33. Patent Owner argues that reliance on Van der Ploeg is flawed because "mere knowledge of the target receptor plus *in vitro* data indicating uptake via that receptor" is insufficient to predict success and further notes particular complications that arise when transitioning from an *in vitro* model to an *in vivo* model. Resp. 25–26. Patent Owner further argues that the grant of orphan drug designation would not be understood by a person of ordinary skill in the art to mean that the therapy had a reasonable expectation to be effective for its intended use. *Id.* at 36 (citing Ex. 2043 ¶ 33). Patent Owner adds that that the standards use by the FDA to grant of orphan drug designation is low and ultimately "unrelated to the standard regulatory requirements for marketing approval or authorization to begin clinical trials." Resp. 34–37 (citing Ex. 2043 ¶¶ 24, 27–28, 42–43; Ex. 2023; Ex. 2027, 368; Ex. 2006; Ex. 2036, 520 (Fig. 1a)).

We are not persuaded. The evidence and arguments cited by Patent Owner highlight the difficulties faced in the development of an enzyme replacement therapy for Pompe disease over a period of decades. For example, in 1973, an infant with Pompe disease was intravenously administered GAA derived from human placenta. Resp. 1–2; Ex. 1010; Ex. 2041 ¶ 31. That patient died. *Id.* Since that time, however, it was discovered that "mannose-6-phosphate receptors are present at the cell surface of myotubes and mediate efficient uptake of lysosomal enzymes containing carbohydrate chains with mannose-6-phosphate residues." Ex.

1032, 90 (citations omitted). The record suggests that it was this discovery, combined with the ability to produce large quantity of enzyme using recombinant technology, which led the field toward human clinical trials. Ex. 1030 ¶ 29 (citing Ex. 1006). Patent Owner does not direct us sufficiently to hurdles that needed to be overcome by the inventors of the '410 patent to achieve the subject matter of claim 1.

Patent Owner's remaining arguments are directed to the predictability of clinical trials in general, which as discussed above in Section II.C.1, we find to be "'nothing more than the routine' application of a well-known problem-solving strategy, . . . 'the work of a skilled [artisan], not of an inventor,'" absent sufficient evidence to the contrary. *Pfizer,* 480 F.3d at 1368.

3. Secondary Considerations

As to secondary considerations, we note that factual inquiries for an obviousness determination include secondary considerations based on evaluation and crediting of objective evidence of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). Notwithstanding what the teachings of the prior art would have suggested to one with ordinary skill in the art at the time of the invention, the totality of the evidence submitted, including objective evidence of nonobviousness, may lead to a conclusion that the claimed invention would not have been obvious to one with ordinary skill in the art. *In re Piasecki*, 745 F.2d 1468, 1471–1472 (Fed. Cir. 1984).

However, such a conclusion requires the finding of a nexus to establish that the evidence relied upon traces its basis to a novel element in the claim and not to something in the prior art. *Institut Pasteur & Universite Pierre et Marie Curie v. Focarino*, 738 F.3d 1337, 1347 (Fed. Cir. 2013).

All types of objective evidence of nonobviousness must be shown to have nexus. *In re GPAC Inc.*, 57 F.3d 1573, 1580 (Fed. Cir. 1995) (nexus generally); *In re Huang*, 100 F.3d 135, 140 (Fed. Cir. 1996) (commercial success); *Rambus Inc. v. Rea*, 731 F.3d 1248, 1256 (Fed. Cir. 2013) (long-felt need); *Muniauction, Inc. v. Thomson Corp.*, 532 F.3d 1318, 1328 (Fed. Cir. 2008) (praise); *Stamps.com Inc. v. Endicia, Inc.*, 437 F. App'x 897, 905 (Fed. Cir. 2011) (skepticism).

Patent Owner argues that several lines of objective evidence (or "secondary considerations") demonstrate the non-obviousness of claim 1. Resp. 56–60. In particular, Patent Owner argues long-felt but unmet need (*id.* at 57–58), skepticism (*id.*), praise (*id.* at 58), and commercial success (*id.* at 59).

Claim 1 recites a biweekly dosing schedule, which Patent Owner contends is a novel element of claim 1. Patent Owner's arguments with regard to each of the secondary considerations, however, fail to establish a nexus between the biweekly feature of claim 1 and any objective evidence of non-obviousness. Rather, the discussion of secondary considerations relates to the merits of the therapeutic compositions of GAA brought to market by Patent Owner. Such compositions, however, were known in the art. *See* discussion in Sections II.C.1 and II.C.2. Accordingly, the objective evidence does not persuade us that claim 1 is non-obvious.

4. Conclusion

In view of the above, we conclude that Petitioner has demonstrated the unpatentability of claim 1 by a preponderance of the evidence.

III. MOTIONS TO EXCLUDE

A. PETITIONER'S MOTION TO EXCLUDE

Petitioner seeks to exclude paragraphs 61, 62, 63, and 66 of the Canfield Declaration, Ex. 2041, because the testimony allegedly is based on insufficient facts or data. Paper 55. Because we do not rely on any of paragraphs 61, 62, 63, and 66 of Ex. 2041 to reach the final decision, we dismiss Petitioner's motion as moot.

B. PATENT OWNER'S MOTION TO EXCLUDE

1. Ex. 1002

Patent Owner seeks to exclude Ex. 1002 as not properly authenticated under Federal Rules of Evidence ("FRE") 901–902. Paper 57, 2–5. Patent Owner further seeks to exclude Ex. 1002 as inadmissible hearsay under FRE 802. *Id.* at 6–7. Patent Owner seeks also to exclude Ex. 1002 under FRE 402 and 403 because it cannot qualify as a printed publication and thus "Exhibit 1002 is irrelevant (FRE 402), can serve only to prejudice Genzyme, [and] is confusing in this context (FRE 403) as it cannot have any bearing on the issue of validity." *Id.* at 7–8.

Federal Rule of Evidence 901(a) states that the authentication requirement is satisfied if the proponent presents "evidence sufficient to support a finding that the item is what the proponent claims it is." Here, Petitioner has presented evidence to authenticate Ex. 1002. That evidence includes an article from the Herald-Sun (Durham, NC) (Ex. 1144)[18]

[18] Exhibit 1144 has the LexisNexis® trade inscription.

published September 3, 1997,[19] discussing the content of the Duke Press Release, and the affidavit of Ms. Beth Nichol, an Investigative Associate at Nichol Investigative Services, LLC, who obtained a copy of the original Duke Press Release from a Duke University library having a Duke University trade inscription (Ex 1182, Ex C). Under FRE 902(6)–(7),[20] Ex. 1144 and Ex. 1182, Ex. C are self-authenticating. Based on the evidence before us, we determine that Ex. 1002 has been authenticated under FRE 901(b)(1), 901(b)(4), 902(6), and 902(7) to warrant its admissibility. The fact that the Duke Press Release was reported in the Herald-Sun newspaper establishes the Duke Press Release as a printed publication.

We further note that Patent Owner fails to identify specifically the portions of Ex. 1002 that it believes to be prejudicial and confusing, or why we would be unable to weigh this evidence without prejudice or confusion. Rather, Patent Owner's objections go more to the weight that Ex. 1002 should be afforded, rather than to its admissibility. A motion to exclude is not the proper vehicle to challenge the sufficiency of evidence. It is within our discretion to assign the appropriate weight to be accorded evidence.

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[19] The Duke Press Release is dated September 2, 1997.

[20] Fed. R. Evid. 902. Evidence that Is Self-Authenticating

The following items of evidence are self-authenticating; they require no extrinsic evidence of authenticity in order to be admitted:

. . . .

(6) Newspapers and Periodicals. Printed material purporting to be a newspaper or periodical.

(7) Trade Inscriptions and the Like. An inscription, sign, tag, or label purporting to have been affixed in the course of business and indicating origin, ownership, or control.

Moreover, we note that there is a strong public policy for making all information filed in an administrative proceeding available to the public, especially in a *inter partes* review, which determines the patentability of claims in an issued patent. It is better to have a complete record of the evidence submitted by the parties than to exclude particular pieces of evidence.

With regard to Patent Owner's hearsay argument, we agree with Petitioner that Ex. 1002 is offered as evidence of what it describes to an ordinary artisan, not for proving the truth of the matters addressed in the document. Paper 64, 8. Accordingly, Ex. 1002 is not hearsay requiring the remedy of exclusion.

Patent Owner further argues that the existence of minor typographical errors in Ex. 1002 prove that Ex. 1002 was not created by Duke University. Paper 66. We are not persuaded. Ex. 1002 appears to be an Internet copy of the original press release, obtainable from a Duke University library. Ex. 1182. Ex. 1002 and the original press release (Ex. 1182, Ex. C) are substantively the same. The presence of minor typographical errors in Ex. 1002 does not persuade us that the content of Ex. 1002 was not created and released by Duke University on September 2, 1997.

2. Exs. 1030 and 1033

Patent Owner seeks to exclude portions of the declarations of Petitioner's experts Dr. Pastores (Ex. 1030) and Dr. Croughan (Ex. 1033), based on their alleged admissions that they lack expertise on in the areas of pre-clinical studies or scaling, and because their testimony allegedly is based on insufficient facts or data. Paper 57, 10–14 (citing FRE 702). Specifically, Patent Owner seeks to exclude paragraphs 25, 26, 31, 38, 39,

44–47, 51–57, 59, 63, 66–71, 74–79, 84–89, 91, 93, and 94 of Ex. 1030 and paragraphs 77–85, 87, 92, 93, 96–100, 102, 108–111, and 114–116 of Ex. 1033.

We have reviewed the cited portions of the testimony provided by Dr. Pastores and Dr. Croughan, and see no basis which would warrant the extreme remedy of exclusion. Patent Owner's objections go to the weight and sufficiency of the testimony, rather than its admissibility. We are capable of discerning from the testimony, and the evidence presented, whether the witness' testimony should be entitled to any weight, either as a whole or with regard to specific issues. We weigh such testimony on an issue-by-issue basis, as appropriate. Furthermore, Patent Owner had the opportunity to address any alleged deficiencies in the testimony of Dr. Pastores and Dr. Croughan in its Patent Owner's Response and we are capable of taking note of those inadequacies and weighing that testimony accordingly.

Thus, we deny Patent Owner's motion seeking to exclude the testimony of Dr. Pastores and Dr. Croughan in this proceeding.

3. Exs. 1021, 1063, 1064, 1071, 1072, 1116, 1174, and 1175

Patent Owner seeks to exclude Exhibits 1021, 1063, 1064, 1071, 1072, 1116, 1174, and 1175 as inadmissible hearsay. Paper 57, 9 and 14–15. Because we do not rely on any of these exhibits to reach the final decision, we dismiss Patent Owner's motion to exclude Exhibit 1021, 1063, 1064, 1071, 1072, 1116, 1174, and 1175 as moot.

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that claim 1 of the '410 patent is determined to be unpatentable;

FURTHER ORDERED that Petitioner's Motion to Exclude is dismissed as moot;

FURTHER ORDERED that Patent Owner's Motion to Exclude is denied-in-part and dismissed-in-part; and

FURTHER ORDERED that because this is a Final Written Decision, parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

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Paper 79
Entered: February 23, 2015

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BIOMARIN PHARMACEUTICAL INC.,
Petitioner,

v.

GENZYME THERAPEUTIC PRODUCTS LIMITED PARTNERSHIP,
Patent Owner.

Case IPR2013-00537
Patent 7,655,226

Before LORA M. GREEN, JACQUELINE WRIGHT BONILLA, and SHERIDAN K. SNEDDEN, *Administrative Patent Judges.*

SNEDDEN, *Administrative Patent Judge*.

FINAL WRITTEN DECISION
35 U.S.C. § 318(a) and 37 C.F.R. § 42.73

I. INTRODUCTION

BioMarin Pharmaceutical Inc. ("Petitioner") filed a Petition to institute an *inter partes* review of claims 1 and 3–6 (Paper 1, "Pet.") of Patent No. 7,655,226 B2 (Ex. 1065, "the '226 patent"). We instituted trial for the challenged claims on the following grounds of unpatentability asserted by Petitioner:

Reference(s)	Basis	Claims challenged
Duke Press Release,[1] Reuser,[2] and Van Hove[3]	§ 103(a)	1 and 3
Duke Press Release, Reuser, Barton,[4] and Van der Ploeg[5]	§ 103(a)	4–6

Decision to Institute (Paper 9, "Dec.") 9.

After institution, the Board of Trustees of Genzyme Therapeutic Products Limited Partnership ("Patent Owner"), filed its Patent Owner's Response ("Resp."). Paper 40. Petitioner filed a Reply (Paper 49, "Reply."). Patent Owner did not file a motion to amend claims.

Petitioner relies upon the declarations of Dr. Gregory M. Pastores

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[1] Duke University, "Duke Obtains FDA Designation for Pompe Disease Therapy", press release dated September 2, 1997, 2 pages (Ex. 1002).

[2] Reuser et al., WO 97/05771, published Feb. 20, 1997 (Ex. 1005).

[3] Van Hove et al., "Purification of recombinant human precursor acid α-glucosidase," 43(3) BIOCHEM. MOL. BIOL. INT. 613-23 (1997) (Ex. 1012).

[4] Barton et al., "Replacement Therapy for Inherited Enzyme Deficiency – Macrophage-Targeted Glucocerebrosidase for Gaucher's Disease," 324 N. ENG. J. MED. 1464-1470 (1991) (Ex. 1004).

[5] Van der Ploeg et al., "Receptor-Mediated Uptake of Acid α-Glucosidase Corrects Lysosomal Glycogen Storage in Cultured Skeletal Muscle," 24(1) PEDIATRIC RESEARCH 90-94 (1988) (Ex. 1032).

("Pastores Declaration") (Ex. 1030) and Dr. Matthew Croughan ("Croughan Declaration") (Ex. 1033) in support of its Petition. Patent Owner relies upon the declaration of William Canfield, M.D., Ph.D. ("Canfield Declaration") (Ex. 2041) in support of its Response.

Petitioner filed a Motion to Exclude certain of Patent Owner's evidence. Paper 53. Patent Owner filed an Opposition (Paper 62), and Petitioner filed a Reply (Paper 64).

Patent Owner filed a Motion to Exclude certain of Petitioner's evidence. Paper 55. Petitioner filed an Opposition (Paper 58), and Patent Owner filed a Reply (Paper 65).

Oral argument was conducted on October 3, 2014. A transcript is entered as Paper 77 ("Tr.").

This Final Written Decision addresses challenges to the patentability of claims 1 and 3–6. Petitioner has shown by a preponderance of the evidence that claims 1 and 3–6 of the '226 patent are unpatentable.

Petitioner's Motion to Exclude is dismissed as moot. Patent Owner's Motion to Exclude is denied-in-part and dismissed-in-part.

A. Related Matters

The parties represent that there are no related matters. Pet. 1; Paper 7; Paper 35.

On the same day Petitioner filed its Petition in this proceeding, however, it also filed two other Petitions seeking *inter partes* review of U.S. Patent No. 7,056,712 (IPR2013-00535) and U.S. Patent No. 7,351,410 (IPR2013-00534), both of which are related to methods of treating Pompe disease

B. The '226 patent (Ex. 1065)

The technology of the patent is enzyme-replacement therapy for patients with Pompe disease, which is caused by deficiency of the lysosomal enzyme acid α-glucosidase ("GAA"). Ex. 1065, 1:18–2:6. The patent discloses a method for treating Pompe disease comprising administering to the patient a therapeutically effective amount of human GAA. *Id.* at 2:30–43. The amount is preferably at least 10 mg of enzyme per kilogram of body weight, which may be administered weekly or two weeks apart. *Id.* at 14:1–18. The claimed treatment results in the arrest or reduction of clinical and biochemical characteristics of Pompe disease, which include, generally, an accumulation of glycogen in various tissues such as heart and skeletal muscle, and more specifically, hypertrophic cardiomyopathy. *Id.* at 13:50–15:43.

C. The Claim

Claims 1 and 6 are the independent claims of the '226 patent, and are reproduced below:

> 1. A method of treating a human patient with Pompe's disease, comprising administering intravenously to the patient a therapeutically effective amount of human acid alpha glucosidase, whereby the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested.
>
> 6. A method of treating a human patient with Pompe's disease, comprising intravenously administering biweekly to the patient a therapeutically effective amount of human acid alpha glucosidase, whereby hypertrophic cardiomyopathy in the patient is reduced and/or arrested.

Claims 3–5 depend from claim 1.

II. DISCUSSION

A. Claim Interpretation

In an *inter partes* review, claim terms in an unexpired patent are interpreted according to their broadest reasonable construction in light of the specification of the patent in which they appear. 37 C.F.R. § 42.100(b); Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,766 (Aug. 14, 2012). Claim terms are given their ordinary and customary meaning, as would be understood by one of ordinary skill in the art in the context of the entire disclosure. *In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007). Any special definition for a claim term must be set forth in the specification with reasonable clarity, deliberateness, and precision. *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

We expressly interpret below only those claim terms that require analysis to resolve arguments related to the patentability of the challenged claims.

1. Construction of the phrase "whereby the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested"

We construe claim 1 to be directed to a method of treating a human patient with Pompe disease. The claimed method comprises a single step: "intravenously administering biweekly to the patient a therapeutically effective amount of human acid alpha glucosidase." Claim 1 further recites the result achieved from the practice of the method recited in claim 1. Specifically, the step of intravenously administering biweekly to the patient a therapeutically effective amount of human GAA results in the reduction in

the concentration of accumulated glycogen in the patient and/or the arrest of further accumulation of glycogen. Thus, the recited whereby clause defines what is achieved from the administration of "a therapeutically effective amount of human acid alpha glucosidase" to a human patient with Pompe disease.

As to what is required in order to achieve the result defined by the whereby clause, Patent Owner proposes a construction that would necessarily include "lysosomal glycogen in the skeletal muscle" within the meaning of the phrase "glycogen in a patient." Resp. 12–16. Patent Owner argues that such a construction is necessary because skeletal muscle must be targeted in order to effectively treat Pompe disease. *Id.* (citing Ex 1162, 62:5–22). Patent Owner further directs our attention to a section of the '226 patent that describes complications and treatment for both the infantile and adult forms of Pompe disease. *Id.* (citing Ex 1065, 15:22–39). For the infantile form, "lysosomal glycogen storage is observed in various tissues, and is most pronounced in heart and skeletal muscle." Ex. 1065, 15:26–28. For the adult form, "skeletal muscle weakness is the major problem; cardiomegaly, hepatomegaly, and macroglossia can be seen, but are rare." *Id.* at 15:48–50.

Petitioner notes that the whereby clause of claim 1 was added during the prosecution of the claim in the parent application, which issued as U.S. Patent No. 7,351,410 (Ex. 1001).[6] Reply, 1–2 (citing Ex. 1022, 3). The paper that introduces the claim language directs the reader to where written description support for the addition of the whereby clause may be found in

[6] The '226 patent is a continuation of US 7,351,410. The claim of US 7,351,410 is challenged by the Petitioner in IPR2013-000534.

the Specification. Ex. 1022, 3. The passage of the Specification cited by the inventor during prosecution for support of the amendment provides as follows:

> When two KO mice were injected 4 times every 6 days (experiment B), a marked decrease of total cellular glycogen was observed in both heart and liver. No effects were observed in skeletal muscle tissues with regard to total glycogen. . . .
>
> The results showed that mice treated 13 weeks with 0.5 mg / mouse (Group A, 3 animals/Group) had an increase of activity in the liver and spleen and decreased levels of glycogen in liver and perhaps in heart. One animal showed increased activity in muscles, although there was no significant decrease of glycogen in muscle.

Ex. 1001, 35:1–3, 31–34. That passage of the Specification summarizes an *in vivo* experiment that resulted in "a marked decrease of total cellular glycogen was observed in both heart and liver," but not in skeletal muscle tissue.

After consideration of the arguments presented by the parties, we do not find that the record supports Patent Owner's construction that would necessarily include "lysosomal glycogen in the skeletal muscle" within the meaning of the phrase "glycogen in a patient." We note that the claim does not recite specific organs or tissue, does not recite any specific form of Pompe disease, and does not require, for example, the patient to experience an increased life-span. The whereby clause merely requires the reduction or arrest of glycogen in the patient. In that regard, the evidence highlighted by Petitioner supports a conclusion that the whereby clause is reasonably interpreted to encompass the scenario where the reduction or arrest of glycogen is observed solely in the heart or liver. Accordingly, we conclude

that the broadest reasonable interpretation of the phrase "glycogen in a patient" does not necessarily include "lysosomal glycogen in the skeletal muscle." Rather, the broadest reasonable interpretation of the phrase "glycogen in a patient," as would be understood by the ordinary artisan in light of the Specification, encompasses lysosomal glycogen in the skeletal muscle, heart, or liver.

B. The Prior Art

1. Summary of Duke Press Release (Ex. 1002)

The Duke Press Release reports the U.S. Food and Drug Administration (FDA) approval of Duke University's application for Orphan Drug Designation for a new therapy for Pompe disease. Ex. 1002; Ex. 1182. The Duke Press Release describes Pompe disease as an inherited deficiency of the enzyme acid alpha glucosidase and provides a discussion of how the therapy, developed at Duke, "will be tested in infants with the most severe symptoms and for whom the disease is fatal." *Id.* With regard to how the therapy would be tested, the Duke Press Release provides as follows:

> The Duke clinical trial will test a genetically engineered form of the enzyme, expressed in a cell line developed in the laboratory of Dr. Y. T. Chen, chief of the Division of Medical Genetics in the department of pediatrics. Initially, the drug will be tested in a small number of Pompe disease infants to evaluate the safety and efficacy of the recombinant enzyme treatment. . . .
>
> Chen, who will lead the clinical trial, anticipates that recombinant enzyme injected into infants will be taken up by their muscle cells and restore normal glycogen levels. This treatment, known as an enzyme replacement therapy, would be required for the rest of these patients' lives.
>
> He said he hopes to expand the treatment to additional Pompe disease patients as safety and efficacy are demonstrated and

> supplies of the enzyme are available. Chen's team at Duke has spent more than five years developing the cell line that produces the recombinant drug.

Id.

2. Summary of Reuser (Ex. 1005)

Reuser discloses the production of phosphorylated lysosomal proteins using transgenic nonhuman mammals for use in enzyme replacement therapy as treatment for lysosomal enzyme deficiencies. Ex. 1005, Abstract, 18:12–14. Reuser expressly discloses Pompe disease[7] as such a lysosomal enzyme deficiency. *Id.* at 2:13–29. With regard to the treatment of Pompe disease, Reuser discloses as follows:

> For lysosomal diseases other than Gaucher disease the evidence suggests that enzyme therapy is most effective when the enzyme being administered is phosphorylated at the 6' position of a mannose side chain group. *For [Pompe disease] this has been tested by intravenously administering purified acid α-glucosidase in phosphorylated and unphosphorylated forms to mice and analyzing uptake in muscle tissue.* The highest uptake was obtained when mannose 6-phosphate-containing enzyme was used (Van der Ploeg et al., Pediat. Res. 28, 344-347 (1990); J. Clin. Invest. 87, 513-518 (1991)).[8]

Id. at 2:35–3:10 (emphasis added).

Reuser expressly identifies GAA as an enzyme useful for production in the disclosed transgenic animal systems. *Id.* at 4:36–37.[9] Specific to

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[7] Also referred to as Glycogen Storage Disease Type II (GSD II).

[8] Citations provided by Petitioner as Ex. 1051 and Ex. 1009, respectively.

[9] Reuser also discloses the use of a stable eukaryotic cell line transfected with the acid α-glucosidase gene for the purposes of producing the human acid α-glucosidase protein. *Id.* at 3:15–18.

human GAA, Reuser discloses a map of several transgenes containing GAA cDNA[10] or genomic DNA,[11] and details experiments in which a mannose 6-phosphate containing human GAA was produced in the milk of transgenic mice. *Id.* at 21:14–28:24. Reuser discloses that the main forms of GAA are the 110/100 kDa precursor, a 95 kDa intermediate, and 76 kDa and 70 kDa mature forms. *Id.* at 9:15–17, 28:19–24.

Reuser contemplates pharmaceutical compositions for use in enzyme replacement therapeutic procedures, and specifically pharmaceutical compositions for intravenous administration. *Id.* at 18:36–20:28. Furthermore, Reuser provides general guidance with regard to dosage and dosage regimen. Specifically, Reuser provides as follows:

> For individuals at risk of lysosomal enzyme deficiency disease, the pharmaceutical composition [sic] are administered prophylactically in an amount sufficient to either prevent or inhibit accumulation of metabolite. An amount adequate to accomplish this is defined as a "therapeutically-" or "prophylactically-effective dose." Such effective dosages will depend on the severity of the condition and on the general state of the patient's health, but will generally range from about 0.1 to 10 mg of purified enzyme per kilogram of body weight.

Id. In the case of Pompe disease, glycogen is the metabolite. *Id.* at 2:13–29.

With regard to dose, Reuser discloses as follows:

> For therapeutic applications, the pharmaceutical compositions are administered to a patient suffering from established disease in an amount sufficient to reduce the concentration of accumulated metabolite and/or prevent or arrest further accumulation of metabolite. For individuals at risk of lysosomal enzyme deficiency disease, the pharmaceutical

[10] Ex. 1005, Fig. 1.

[11] Ex. 1005, Fig. 2.

> composition are administered prophylactically in an amount sufficient to either prevent or inhibit accumulation of metabolite. An amount adequate to accomplish this is defined as a "therapeutically-" or "prophylactically-effective dose." Such effective dosages will depend on the severity of the condition and on the general state of the patient's health, but will generally range from about 0.1 to 10 mg of purified enzyme per kilogram of body weight.

Id. at 20:15–28.

3. Summary of Van Hove (Ex. 1012)

Van Hove discloses a purification of large quantities of recombinant precursor GAA for the purposes of enzyme replacement therapy in Pompe disease. Ex. 1012, 613. In particular, Van Hove provides as follows:

> Large quantities of recombinant enzyme are required for in vivo experimentation in the animal model, and eventually for use in medicine. A method amenable to scale up is now needed to efficiently purify recombinant precursor enzyme from tissue culture medium. The preferred method should result in a high level of purification with a considerable yield, while preserving the mannose-6-phosphorylation of the protein required for efficient lysosomal targeting. It should be highly reproducible, avoid toxic buffers, preferably use commercially available gels on standard equipment. We aimed to develop such a purification method as described below.

Id. at 614. Precursor GAA is the 110 kDa form of the enzyme. *Id.* at 617.

4. Summary of Barton (Ex. 1004)

Barton describes a clinical trial in which patients with Gaucher Disease[12] were administered glucocerebrosidase for enzyme replacement

[12] Gaucher disease is a lysosomal storage disorder caused by an insufficiency of glucocerebrosidase. Ex. 1004, 1464.

therapy on a biweekly intravenous administration schedule. Ex. 1004, Abstract.

5. Summary of Van der Ploeg (Ex. 1032)

Van der Ploeg describes an *in vitro* study using cultured skeletal muscle cells from a patient with Pompe disease. Ex 1032, Abstract. Cultured skeletal muscle cells were incubated with GAA containing mannose-6-phosphate purified from human urine. *Id.* Van der Ploeg reports that the "[e]fficient uptake of acid α-glucosidase was achieved by using the mannose-phosphate receptor on the cell surface as a target for an enzyme precursor with phosphorylated high-mannose types carbohydrate chains purified from human urine." *Id.* The enzyme was reported to have a half-life of 6–9 days. *Id.* at 91.

C. Obviousness of Original Claims

1. Obviousness of Claims 1 and 3 over the Combination of Duke Press Release, Reuser, and Van Hove

Petitioner contends that claims 1 and 3 are obvious over the combination of Duke Press Release 1997, Reuser, and Van Hove. Pet. 41–44. In our Decision to Institute, we found that the combination of Duke Press Release, Reuser, and Van Hove disclosed each element of claims 1 and 3. Dec. 7–8. Patent Owner does not contend that the combination of references fail to address each element of the claims. Rather, Patent Owner contends that a person of ordinary skill in the art would not have had a reasonable expectation of successfully reducing or arresting further accumulation of glycogen in a patient's muscle cells. Resp. 35–40.

We are not persuaded. First, Patent Owner's argument is premised on a claim construction that would require the phrase "glycogen in the patient" in claim 1 necessarily to include lysosomal glycogen in skeletal muscle. *Id.* at 14–16. As discussed above in Section II.A.1, however, we do not adopt Patent Owner's proposed claim construction in this regard. As such, we do not understand Patent Owner's arguments with regard to a reasonable expectation of success to be applicable to the question of whether or not a person of ordinary skill in the art would have had a reasonable expectation of successfully reducing or arresting further accumulation of glycogen in a patient's other tissues such as heart and liver.

To the extent that Patent Owner's arguments are applicable under our claim construction, we note that a reasonable expectation of success does not require absolute predictability. *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). The fact that a suggested dose (as taught in Reuser) and dosing schedule had not been established yet as safe and effective in human clinical trials at the time of invention does not demand a conclusion of nonobviousness. While we recognize that there would have been some degree of unpredictability for the successful treatment of Pompe disease from the administration of GAA, the preponderance of evidence of record indicates that all that remained to be achieved over the prior art was the determination that a specific dose within a previously suggested dose range, and its corresponding dosing schedule, would have been safe and effective for the treatment of human patients.

By December 7, 1998, the field related to the development of an enzyme replacement therapy for the treatment of Pompe disease had developed to the point at which 1) it was recognized that GAA must be post-

translationally modified with mannose-6-phosphate to promote cellular uptake through a mannose-6-phosphate receptor *in vitro*;[13] 2) *in vivo* studies had been performed in which GAA containing mannose-6-phosphate was intravenously administered to mice[14] and Japanese Quail;[15] 3) it was known that mannose 6-phosphate containing human GAA could be produced in the milk of transgenic animals;[16] and 4) the FDA was granting applications for orphan drug designation for enzyme replacement therapy for Pompe disease using recombinant GAA.[17] Thus, this is not a case where the prior art teaches merely to pursue a "general approach that seemed to be a promising field of experimentation" or "gave only general guidance as to the particular form of the claimed invention or how to achieve it." *O'Farrell*, 853 F.2d at 903; *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1167 (Fed. Cir. 2006).

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[13] Ex. 1005 at 2:35–3:10.

[14] Ex. 1009 presents data suggesting that GAA containing mannose 6-phosphate is taken up in the skeletal muscle and heart of mice after intravenous administration.

[15] Ex. 1007 presents data suggesting that the intravenous administration of human GAA to GAA-deficient Japanese reduced glycogen levels in the heart, liver and muscle and produced muscle improvement. Ex. 1007, Abstract. The authors conclude that "[t]hese data also suggest enzyme replacement with recombinant human GAA is a promising therapy for human Pompe disease." *Id.*

[16] Ex. 1005 at 21:14–28:24.

[17] Ex. 1002; Ex. 1182. We further note that the FDA application process requires an applicant to provide "enough information to establish a medically plausible basis for expecting the drug to be effective in the rare disease." Ex. 1029. Furthermore, as stated by Dr. Canfield, "a [skilled artisan] would know, and would understand from Ex 1002, that the purpose of the proposed clinical trial would be to evaluate whether the administered enzyme was safe and effective in humans and to determine the appropriate dose." Ex 2041 ¶ 81 (citing Ex 1002, 2).

Finally, we note that the absence in the record of evidence identifying a difference between the prior art and the subject matter of the claims further persuades us that no more than routine processes were needed to achieve the results recited in claim 1. For example, the absence of any discussion with regard to unexpected or superior results associated with any feature of claims 1 or 3 further persuades us that the claimed subject matter was a product of routine clinical trial processes. That is, the prior art brought the subject matter of the claims within the technical grasp of a person of ordinary skill in the art rendering it obvious, absent objective evidence of nonobviousness. *Pfizer*, 480 F.3d at 1344 ("[O]bviousness cannot be avoided simply by a showing of some degree of unpredictability in the art so long as there was a reasonable probability of success.").

In view of the above, we conclude that a person of ordinary skill in the art would have had a reasonable expectation of success at the time the invention was made. What remained was the execution of human clinical trials, arguably "routine" to a person of ordinary skill in the art, to verify the expectation that a specific dosage (within a previously suggested dosage range) and corresponding dosage regimen would have been safe and effective. *Cf. Pfizer*, 480 F.3d at 1367 ("[E]xperiments used by Pfizer's scientists to verify the physicochemical characteristics of each salt are not equivalent to the trial and error procedures often employed to discover a new compound where the prior art gave no motivation or suggestion to make the new compound nor a reasonable expectation of success."); *Velander v. Garner*, 348 F.3d 1359, 1368 (Fed. Cir. 2003) (stating that one skilled in the art would view variability in producing fibrinogen in transgenic mammals as

evidence that "expense, time and effort" would be involved did not equate to a conclusion that success was unlikely).

2. Obviousness of Claims 4–6 over the Combination of Duke Press Release, Reuser, Barton, and Van der Ploeg

a. Obviousness of the recited dose and dosing schedule

Claim 4 depends directly from claim 1 and requires weekly administration of GAA. Independent claim 6 differs from claim 1, *inter alia*, in that the method of claim 6 requires biweekly administration of the enzyme. Petitioner relies on Barton (Ex. 1004) and Van der Ploeg (Ex. 1032) to reach the "weekly" and "biweekly" elements of claims 4 and 6.

Claim 5 depends from claim 4 and requires that the therapeutically effective amount of human GAA is at least 10 mg/kg body weight of the patient. Petitioner relies on Reuser to meet that limitation, as Reuser discloses a dosage range from 0.1 to 10 mg/kg of purified enzyme per kilogram of body weight. Pet. 47 (citing Ex. 1005, 20:27–28).

Petitioner argues that "[d]etermination of how much and how often to administer the enzyme . . . is a matter of routine optimization" and that "[t]he '226 patent is claiming the result of a typical drug development pathway based on previous testing, such as *in vitro* and *in vivo* animal model studies." Pet. 4–5; *see also, id.* at 9 ("All the inventors of the van Bree '226 patent did was to follow a typical drug development pathway laid out in the prior art, where the use of GAA to successfully treat Pompe disease in a human patient was a predictable outcome based on previous *in vitro* and *in vivo* studies.").

Patent Owner makes several arguments related to the dose and dosing schedule elements of the claims. First, Patent Owner contends that a person

of ordinary skill in the art would not have had motivation to combine Barton with Duke Press Release, Reuser, and Van der Ploeg due to the significant differences between Gaucher disease and Pompe disease. Resp. 41.

Second, Patent Owner argues that reliance on Van der Ploeg is flawed because "mere knowledge of the target receptor plus *in vitro* data indicating uptake via that receptor" is insufficient to predict success and further notes particular complications that arise when transitioning from an *in vitro* model to an *in vivo* model. *Id.* at 23–24.

Patent Owners remaining arguments are directed to the predictability of clinical trials in general. *Id.* at 44–46. Additionally, Patent Owner argues that the grant of orphan drug designation disclosed in Duke Press Release would not be understood by a person of ordinary skill in the art to mean that the therapy had a reasonable expectation to be effective for its intended use. *Id.* at 32–35. Patent Owner adds that that the standards used by the FDA to grant of orphan drug designation is low and ultimately "unrelated to the standard regulatory requirements for marketing approval or authorization to begin clinical trials." *Id.* at 33–34 (citing Ex 2043 ¶¶ 24, 27–28, 42–43; Ex 2023; Ex 2027, 368; Ex 2006; Ex. 2036, 520 (Fig. 1a)).

After consideration of the evidence and arguments summarized above, we find that Petitioner has the better position. We recognize that the record shows that human clinical trials were not initiated prior to December 7, 1998, the priority date of the '226 patent. The record shows also that an ordinary artisan would have understood that to treat Pompe disease effectively using GAA, sufficient quantities of enzyme would have to reach the patient's muscle cells, which could potentially require high doses that could introduce safety and efficacy hurdles resolvable only with human

clinical trials.[18] Resp. 55–57 (citing Ex 2041 ¶ 105; Ex 2042 ¶¶ 67-68, 86, 99, 121-122; Ex 1162, 63:14-64:13, 67:2-11; Ex. 1030 ¶ 75; Ex 1011). Accordingly, a person of ordinary skill in the art could not have predicted with absolute certainty that a safe and effective dosing regimen for using GAA in a method of treating Pompe disease could be achieved.[19]

Despite this recognized difficultly, however, we are persuaded that, under the facts of this case, a person of ordinary skill would have been motivated to pursue the clinical development of the therapy disclosed in Reuser. *See* Section II.B.2 for summary of Rueser. Given that Reuser discloses a pharmaceutical composition containing GAA, a dose range that includes the recited dose range of 1–10 mg of enzyme per kilogram of body weight of the individual, a method of making the composition using transgenic animals, and a method of using the composition in an enzyme replacement therapy for the treatment of Pompe disease, what remained to

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[18] As discussed in Section II.A.1, we do not interpret the claim to necessarily require the reduction or arrest of stored glycogen in skeletal muscle, but understand that achieving such a result is important for the complete treatment of both forms of Pompe disease. Nonetheless, we include the concept of reduction or arrest of stored glycogen in skeletal muscle in our analysis as we do not find its inclusion to hinder our analysis. That is, whether we include or exclude the result of reduction or arrest of stored glycogen in skeletal muscle within the scope of the claims, our conclusions remain the same, albeit achievement of the reduction of glycogen in the heart and liver appear to be less controversial, and thus the evidence suggests there was a greater expectation of success with regard to these tissues.

[19] We recognize that "absolute predictability of success" is not the criterion "[f]or obviousness under § 103, all that is required is a reasonable expectation of success." *O'Farrell*, 853 F.2d at 903.

be achieved to arrive at the claimed subject matter was the selection of a specific dose and dosing schedule for a treatment regimen. Pet. 4–5.

For example, in 1973, an infant with Pompe disease was intravenously administered GAA derived from human placenta. Resp. 1–2; Ex. 1010; Ex. 2041 ¶ 31. That patient died. *Id.* Since that time, however, it was discovered that "mannose-6-phosphate receptors are present at the cell surface of myotubes and mediate efficient uptake of lysosomal enzymes containing carbohydrate chains with mannose-6-phosphate residues." Ex. 1032, 90 (citations omitted). The record suggests that it was this discovery, combined with the ability to produce large quantity of enzyme using recombinant technology, which led the field toward human clinical trials. Ex. 1030 ¶ 29 (citing Ex. 1006). Patent Owner does not direct us sufficiently to hurdles that needed to be overcome by the inventors of the '226 patent to achieve the claimed subject matter.

Thus, our decision rests on the answer to the question of whether the dose and dosing schedule recited in the claims were nothing more than the result of routine optimization that would have been obvious to one of ordinary skill in the art. We conclude that a preponderance of the evidence establishes that the selection of the dose and dosing schedule would have been a routine optimization of the therapy outlined in Reuser (Ex. 1005, 18:36–20:28), which would have been achievable through the use of standard clinical trial procedures (Ex. 1030 ¶¶ 74–90). Stated differently, the subject matter of the claims was disclosed in the prior art and the experimentation needed to confirm the successful application of the method disclosed in the prior art was "'nothing more than the routine' application of a well-known problem-solving strategy, . . . 'the work of a skilled [artisan],

not of an inventor.'" *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1368 (Fed. Cir. 2007) (quoting *Merck & Co. v. Biocraft Labs., Inc.*, 874 F.2d 804, 809 (Fed. Cir. 1989); *DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1371 (Fed. Cir. 2006); *see also In re Aller*, 220 F.2d 454, 456 (CCPA 1955) ("[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation."); *In re Boesch*, 617 F.2d 272, 276 (CCPA 1980) ("[D]iscovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art."). The motivation to optimize the therapy disclosed in Reuser "flows from the 'normal desire of scientists or artisans to improve upon what is already generally known.'" *Pfizer,* 480 F.3d at 1348 (quoting *In re Peterson*, 315 F.3d 1325, 1330 (Fed. Cir. 2003)).

We further note that this is not a case where there were "numerous parameters" to try. *Pfizer*, 480 F.3d at 1364 (citing *Medichem*, 437 F.3d at 1165 ("to have a reasonable expectation of success, one must be motivated to do more than merely to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.") (internal quotations omitted)). Rather, we are persuaded by Dr. Pastores' testimony that the knowledge in the art regarding the treatment of Pompe disease with human GAA would have provided the motivation to select a suitable dose and dosing schedule (Ex. 1030 ¶ 38), would have been informed by the clinical experience with Gaucher disease (*id.* at ¶ 74 (citing Ex. 1004, 1056, 1057)), and that, because "it was well known that any

enzyme replacement therapy for Pompe disease would be required for the rest of a patient's life, . . . repeated spaced administration of GAA to patients would be immediately understood upon reading [Reuser]" (*id.* at ¶ 58).

b. Reducing or Arresting Hypertrophic Cardiomyopathy

Independent claim 6 further differs from claim 1, *inter alia*, in that the method of claim 6 requires hypertrophic cardiomyopathy in the patient to be reduced and/or arrested, as oppose to the concentration of accumulated glycogen in the patient to be reduced and/or arrested. The preponderance of evidence shows that the concentration of accumulated glycogen in the patient is related to the condition of hypertrophic cardiomyopathy. *See also,* Ex 2041, ¶ 24 ("[Pompe disease] patients develop accumulation of glycogen in the heart and skeletal muscle, which results in progressive deterioration of the heart muscle (cardiomyopathy) and generalized muscle weakness"). As such, we conclude that if the concentration of accumulated glycogen in the patient is arrested, for example, then the condition caused by this accumulation is also necessarily arrested, at least in some fashion. Accordingly, we conclude that independent claim 6 is unpatentable for the reasons set forth above with regard to claim 1.

3. Secondary Considerations

As to secondary considerations, we note that factual inquiries for an obviousness determination include secondary considerations based on evaluation and crediting of objective evidence of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). Notwithstanding what the teachings of the prior art would have suggested to one with ordinary skill in the art at the time of the invention, the totality of the evidence submitted,

including objective evidence of nonobviousness, may lead to a conclusion that the claimed invention would not have been obvious to one with ordinary skill in the art. *In re Piasecki*, 745 F.2d 1468, 1471–1472 (Fed. Cir. 1984).

However, such a conclusion requires the finding of a nexus to establish that the evidence relied upon traces its basis to a novel element in the claim and not to something in the prior art. *Institut Pasteur & Universite Pierre et Marie Curie v. Focarino*, 738 F.3d 1337, 1347 (Fed. Cir. 2013). All types of objective evidence of nonobviousness must be shown to have nexus. *In re GPAC Inc.*, 57 F.3d 1573, 1580 (Fed. Cir. 1995) (nexus generally); *In re Huang*, 100 F.3d 135, 140 (Fed. Cir. 1996) (commercial success); *Rambus Inc. v. Rea*, 731 F.3d 1248, 1256 (Fed. Cir. 2013) (long-felt need); *Muniauction, Inc. v. Thomson Corp.*, 532 F.3d 1318, 1328 (Fed. Cir. 2008) (praise); *Stamps.com Inc. v. Endicia, Inc.*, 437 F. App'x 897, 905 (Fed. Cir. 2011) (skepticism).

Patent Owner argues that several lines of objective evidence (or "secondary considerations") demonstrate the non-obviousness of the challenged claims. Resp. 56–60. In particular, Patent Owner argues long-felt but unmet need, skepticism, praise, and commercial success. *Id.* Patent Owner's arguments with regard to each of the secondary considerations, however, fail to establish a nexus between any feature of the claims and any asserted objective evidence of non-obviousness. Rather, the discussion of secondary considerations relates to the merits of the therapeutic compositions of GAA brought to market by Patent Owner. Such compositions, however, were known in the art. *See* discussion in Sections II.C.1 and II.C.2. Accordingly, the objective evidence does not persuade us that claim 1 and 3–6 are non-obvious.

4. Conclusion

In view of the above, we conclude that Petitioner has demonstrated the unpatentability of claims 1 and 3–6 by a preponderance of the evidence.

III. MOTIONS TO EXCLUDE

A. PETITIONER'S MOTION TO EXCLUDE

Petitioner seeks to exclude paragraphs 61, 62, 63, and 66 of the Canfield Declaration, Ex. 2041, because the testimony allegedly is based on insufficient facts or data. Paper 55. Because we do not rely on any of paragraphs 61, 62, 63, and 66 of Ex. 2041 to reach the final decision, we dismiss Petitioner's motion as moot.

B. PATENT OWNER'S MOTION TO EXCLUDE

1. Ex. 1002

Patent Owner seeks to exclude Ex. 1002 as not properly authenticated under Federal Rules of Evidence ("FRE") 901–902. Paper 55, 2–5. Patent Owner further seeks to exclude Ex. 1002 as inadmissible hearsay under FRE 802. *Id.* at 6–7. Patent Owner seeks also to exclude Ex. 1002 under FRE 402 and 403 because it cannot qualify as a printed publication and thus "Exhibit 1002 is irrelevant (FRE 402), can serve only to prejudice Genzyme, [and] is confusing in this context (FRE 403) as it cannot have any bearing on the issue of validity." *Id.* at 7–8.

Federal Rule of Evidence 901(a) states that the authentication requirement is satisfied if the proponent presents "evidence sufficient to support a finding that the item is what the proponent claims it is." Here, Petitioner has presented evidence to authenticate Ex. 1002. That evidence

includes an article from the Herald-Sun (Durham, NC) (Ex. 1144)[20] published September 3, 1997,[21] discussing the content of the Duke Press Release, and the affidavit of Ms. Beth Nichol, an Investigative Associate at Nichol Investigative Services, LLC, who obtained a copy of the original Duke Press Release from a Duke University library having a Duke University trade inscription (Ex 1182, Ex C). Under FRE 902(6)–(7),[22] Ex. 1144 and Ex. 1182, Ex. C are self-authenticating. Based on the evidence before us, we determine that Ex. 1002 has been authenticated under FRE 901(b)(1), 901(b)(4), 902(6), and 902(7) to warrant its admissibility. The fact that the Duke Press Release was reported in the Herald-Sun newspaper establishes the Duke Press Release as a printed publication.

We further note that Patent Owner fails to identify specifically the portions of Ex. 1002 that it believes to be prejudicial and confusing, or why we would be unable to weigh this evidence without prejudice or confusion. Rather, Patent Owner's objections go more to the weight that Ex. 1002 should be afforded, rather than to its admissibility. A motion to exclude is

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[20] Exhibit 1144 has the LexisNexis® trade inscription.

[21] The Duke Press Release is dated September 2, 1997.

[22] Fed. R. Evid. 902. Evidence that Is Self-Authenticating

The following items of evidence are self-authenticating; they require no extrinsic evidence of authenticity in order to be admitted:

. . . .

(6) Newspapers and Periodicals. Printed material purporting to be a newspaper or periodical.

(7) Trade Inscriptions and the Like. An inscription, sign, tag, or label purporting to have been affixed in the course of business and indicating origin, ownership, or control.

not the proper vehicle to challenge the sufficiency of evidence. It is within our discretion to assign the appropriate weight to be accorded evidence.

Moreover, we note that there is a strong public policy for making all information filed in an administrative proceeding available to the public, especially in a *inter partes* review, which determines the patentability of claims in an issued patent. It is better to have a complete record of the evidence submitted by the parties than to exclude particular pieces of evidence.

With regard to Patent Owner's hearsay argument, we agree with Petitioner that Ex. 1002 is offered as evidence of what it describes to an ordinary artisan, not for proving the truth of the matters addressed in the document. Paper 65, 8. Accordingly, Ex. 1002 is not hearsay requiring the remedy of exclusion.

Patent Owner further argues that the existence of minor typographical errors in Ex. 1002 prove that Ex. 1002 was not created by Duke University. Paper 65. We are not persuaded. Ex. 1002 appears to be an Internet copy of the original press release, obtainable from a Duke University library. Ex. 1182. Ex. 1002 and the original press release (Ex. 1182, Ex. C) are substantively the same. The presence of minor typographical errors in Ex. 1002 does not persuade us that the content of Ex. 1002 was not created and released by Duke University on September 2, 1997.

2. Exs. 1030 and 1033

Patent Owner seeks to exclude portions of the declarations of Petitioner's experts Dr. Pastores (Ex. 1030) and Dr. Croughan (Ex. 1033), based on their alleged admissions that they lack expertise on in the areas of pre-clinical studies or scaling, and because their testimony allegedly is based

on insufficient facts or data. Paper 55, 10–14 (citing FRE 702). Specifically, Patent Owner seeks to exclude paragraphs 25, 26, 31, 38, 39, 44–47, 51–57, 59, 63, 66–71, 74–79, 84–89, 91, 93, and 94 of Ex. 1030 and paragraphs 77–85, 87, 92, 93, 96–100, 102, 108–111, and 114–116 of Ex. 1033.

We have reviewed the cited portions of the testimony provided by Dr. Pastores and Dr. Croughan, and see no basis which would warrant the extreme remedy of exclusion. Patent Owner's objections go to the weight and sufficiency of the testimony, rather than its admissibility. We are capable of discerning from the testimony, and the evidence presented, whether the witness' testimony should be entitled to any weight, either as a whole or with regard to specific issues. We weigh such testimony on an issue-by-issue basis, as appropriate. Furthermore, Patent Owner had the opportunity to address any alleged deficiencies in the testimony of Dr. Pastores and Dr. Croughan in its Patent Owner's Response and we are capable of taking note of those inadequacies and weighing that testimony accordingly.

Thus, we deny Patent Owner's motion seeking to exclude the testimony of Dr. Pastores and Dr. Croughan in this proceeding.

3. Exs. 1021, 1063, 1064, 1071, 1072, 1116, 1174, and 1175

Patent Owner seeks to exclude Exhibits 1021, 1063, 1064, 1071, 1072, 1116, 1174, and 1175 as inadmissible hearsay. Paper 55, 9 and 14–15. Because we do not rely on any of these exhibits to reach the final decision, we dismiss Patent Owner's motion to exclude Exhibit 1021, 1063, 1064, 1071, 1072, 1116, 1174, and 1175 as moot.

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that claims 1 and 3–6 of the '226 patent is determined to be unpatentable;

FURTHER ORDERED that Petitioner's Motion to Exclude is dismissed as moot;

FURTHER ORDERED that Patent Owner's Motion to Exclude is denied-in-part and dismissed-in-part; and

FURTHER ORDERED that because this is a Final Written Decision, parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

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US007351410B2

(12) United States Patent
van Bree et al.

(10) Patent No.: US 7,351,410 B2
(45) Date of Patent: Apr. 1, 2008

(54) **TREATMENT OF POMPE'S DISEASE**

(75) Inventors: **Johannes B. M. M. van Bree**, Nieuw-Vennep (NL); **Edna H. G. Venneker**, Saturnushof 15 (NL); **David P. Meeker**, Concord, MA (US)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 328 days.

(21) Appl. No.: **10/611,598**

(22) Filed: **Jun. 30, 2003**

(65) **Prior Publication Data**

US 2004/0081645 A1 Apr. 29, 2004

Related U.S. Application Data

(63) Continuation of application No. 09/454,711, filed on Dec. 6, 1999, now abandoned.

(60) Provisional application No. 60/111,291, filed on Dec. 7, 1998.

(51) **Int. Cl.**
A61K 38/46 (2006.01)
A61K 38/43 (2006.01)

(52) **U.S. Cl.** **424/94.61**; 424/94.1; 424/94.6; 435/183; 435/200; 435/201

(58) **Field of Classification Search** 424/94.61, 424/94.6, 94.1; 435/201, 200, 183
See application file for complete search history.

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(Continued)

Primary Examiner—Ruth A Davis
(74) *Attorney, Agent, or Firm*—Hamilton, Brook, Smith & Reynolds, P.C.

(57) **ABSTRACT**

The invention provides methods of treating Pompe's disease using human acid alpha glucosidase. A preferred treatment regime comprises administering greater than 10 mg/kg body weight per week to a patient.

1 Claim, 7 Drawing Sheets

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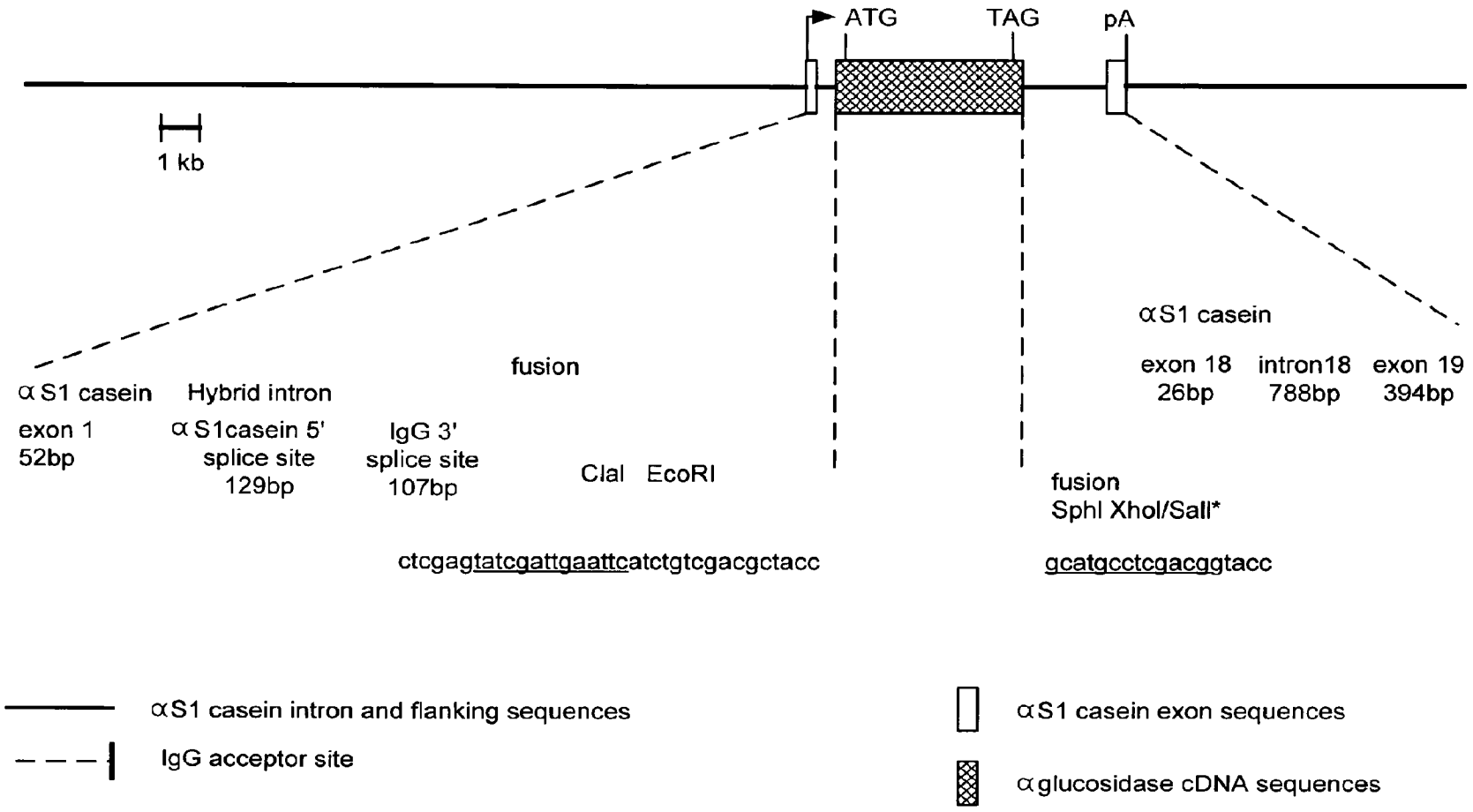


FIG. 1

FIG. 2A

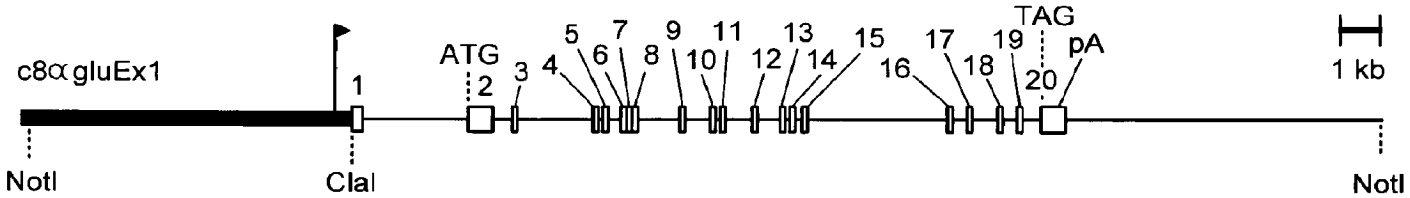


FIG. 2B

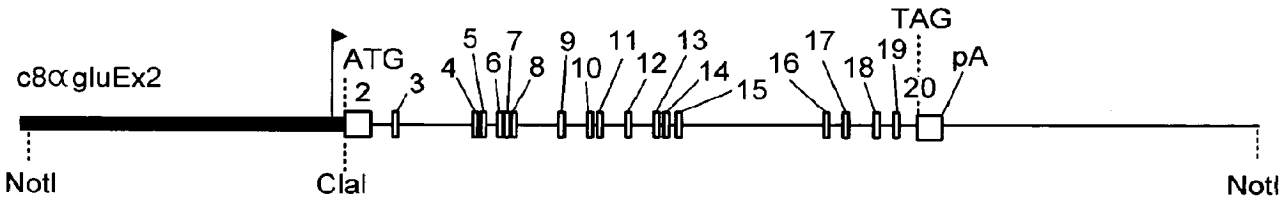
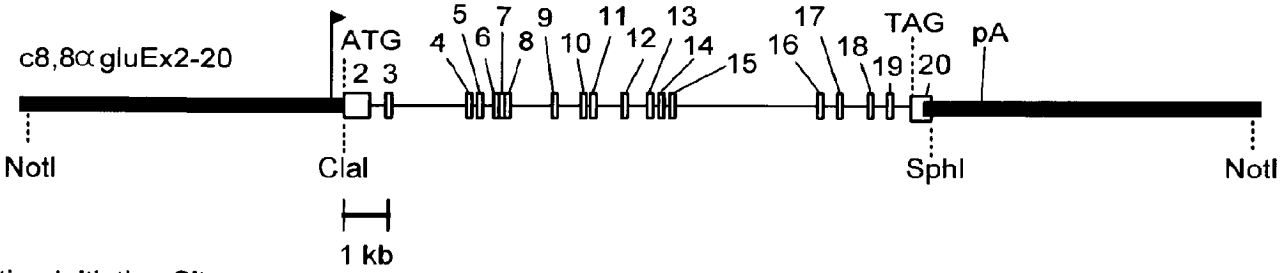


FIG. 2C



Transcription Initiation Site

α_{s1} casein sequence, promoter or 3' untranslated region

2 3 The boxes represent the exons in the α - glucosidase sequence, the thin line represents the intron sequences. The numbers above the boxes are the exon numbers.

pA = polyadenylation signal

ATG = translation initiation site

TAG = translation stop codon

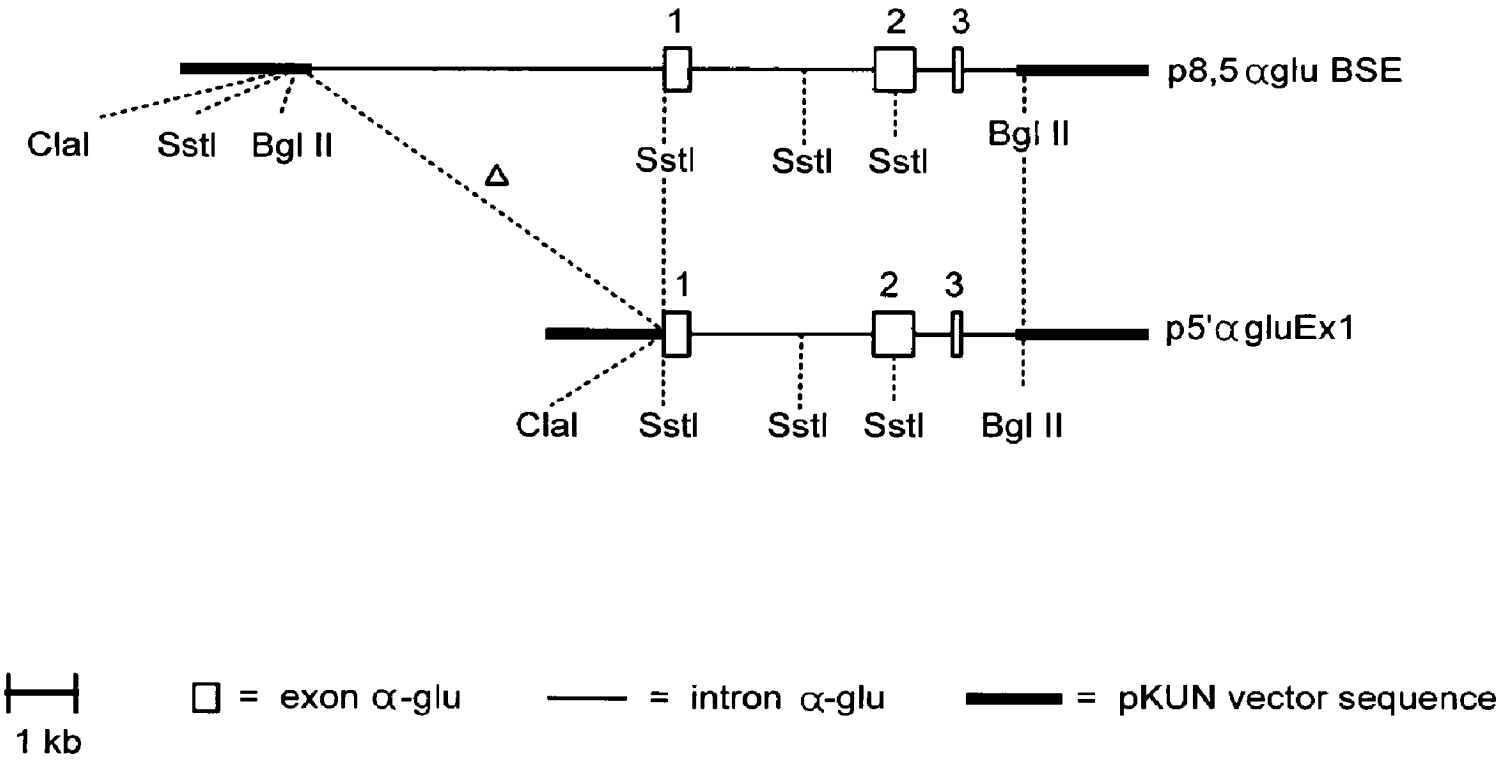


FIG. 3A

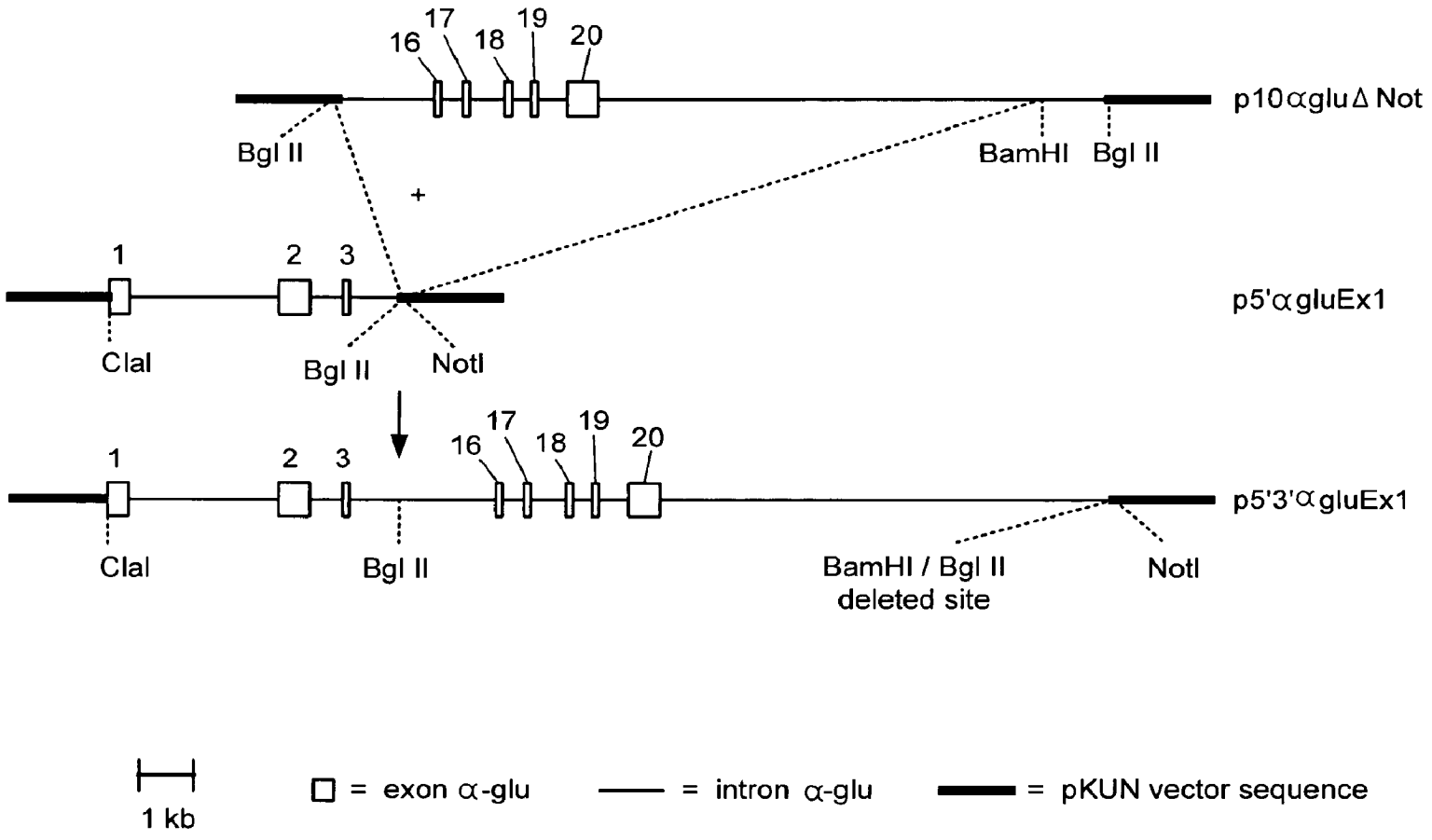


FIG. 3B

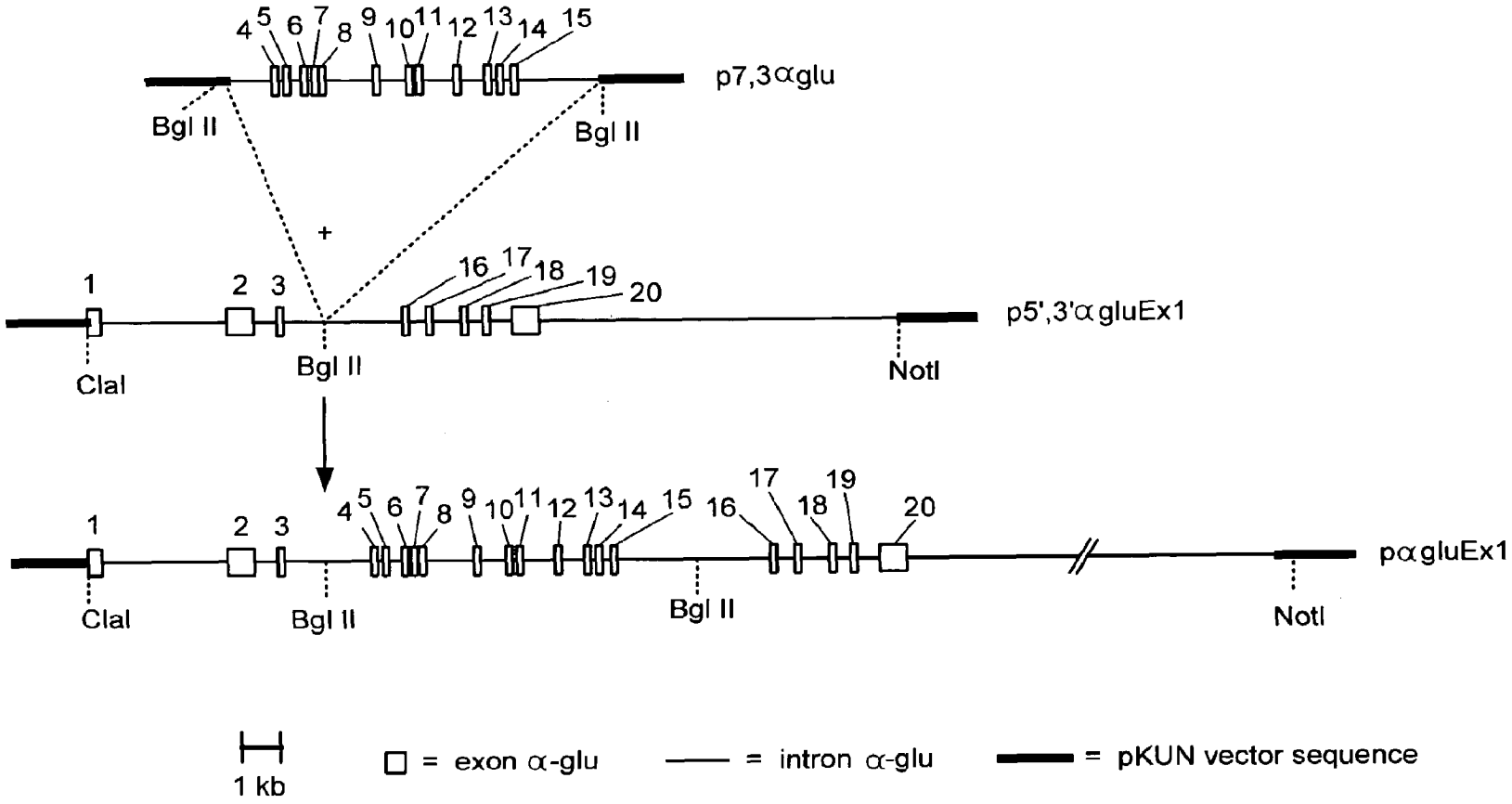


FIG. 3C

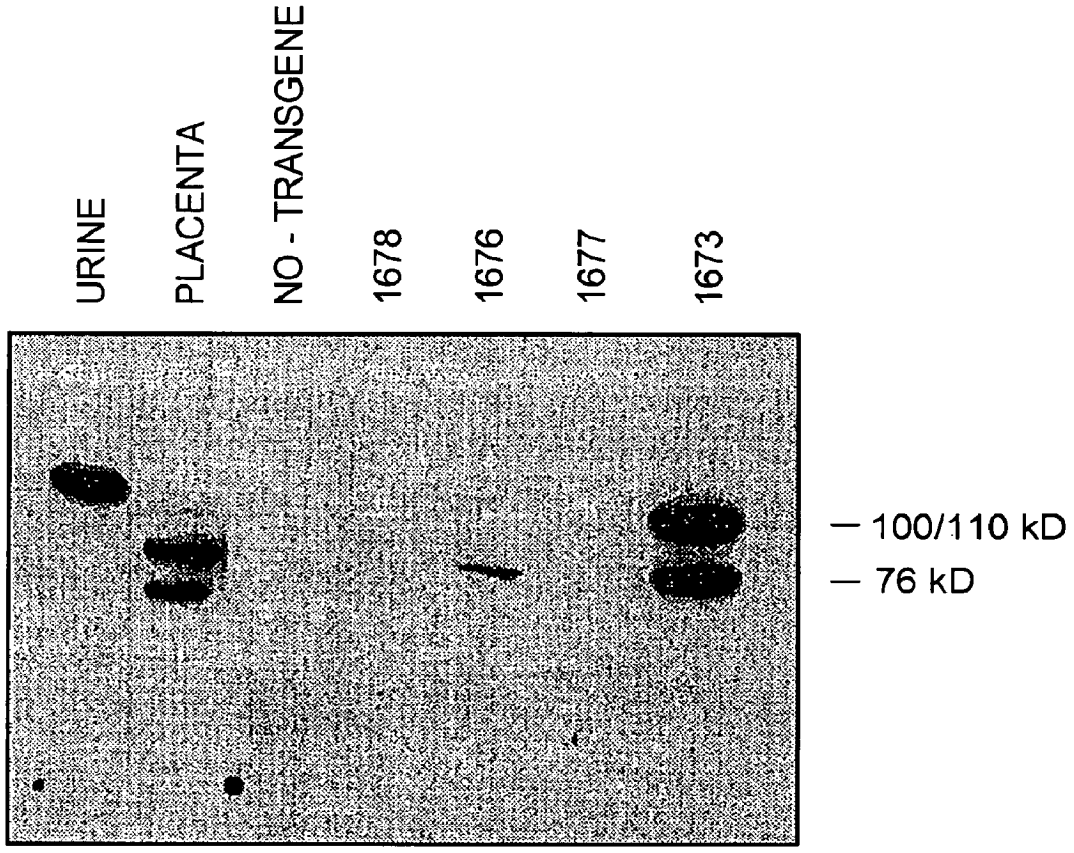


FIG. 4A

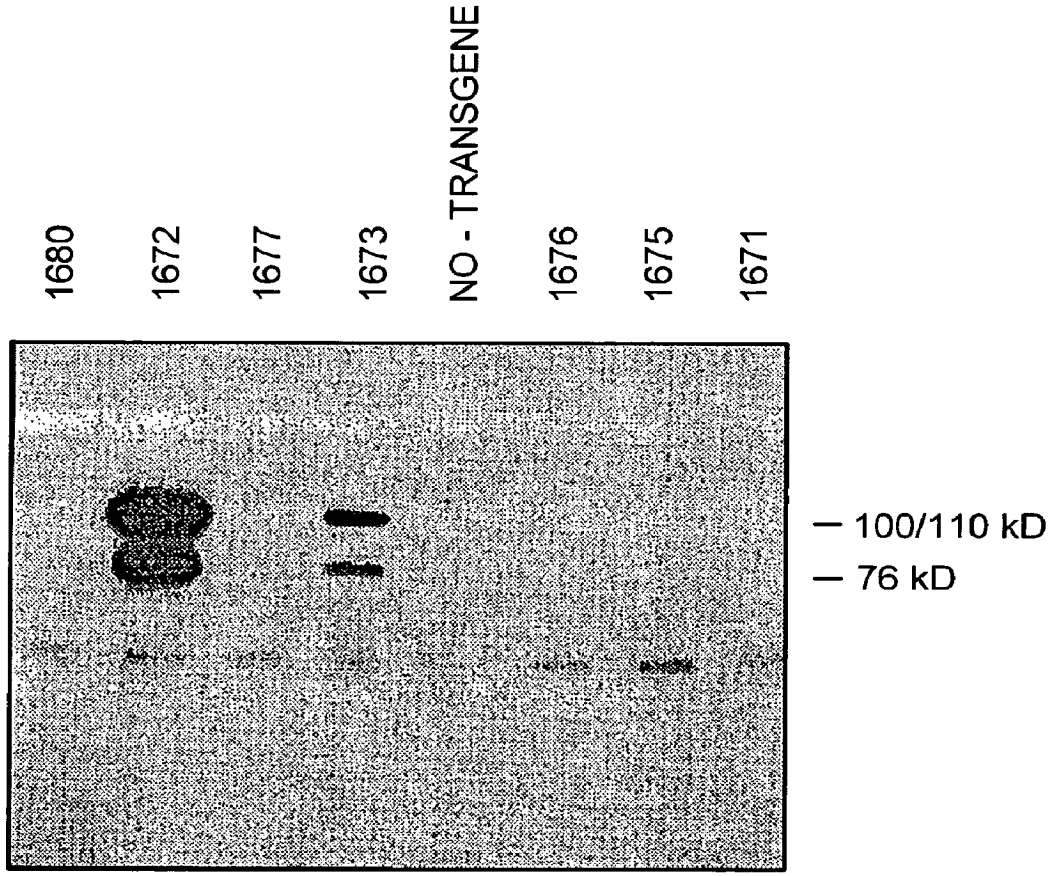


FIG. 4B

TREATMENT OF POMPE'S DISEASE

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application is a continuation of U.S. application Ser. No. 09/454,711, filed Dec. 6, 1999 now abandoned, which claims the benefit of U.S. Provisional Application No. 60/111,291 filed Dec. 7, 1998, both of which are incorporated by reference in their entirety for all purposes. The present application is related to U.S. application Ser. No. 08/700,760 filed Jul. 29, 1996, now U.S. Pat. No. 6,118,045, which claims the benefit of U.S. Provisional Application No. 60/001,796, filed Aug. 2, 1995, both of which are incorporated by reference in their entirety for all purposes.

TECHNICAL FIELD

The present invention resides in the fields of recombinant genetics, and medicine, and is directed to enzyme-replacement therapy of patients with Pompe's disease.

BACKGROUND OF THE INVENTION

Like other secretory proteins, lysosomal proteins are synthesized in the endoplasmic reticulum and transported to the Golgi apparatus. However, unlike most other secretory proteins, the lysosomal proteins are not destined for secretion into extracellular fluids but into an intracellular organelle. Within the Golgi, lysosomal proteins undergo special processing to equip them to reach their intracellular destination. Almost all lysosomal proteins undergo a variety of posttranslational modifications, including glycosylation and phosphorylation via the 6' position of a terminal mannose group. The phosphorylated mannose residues are recognized by specific receptors on the inner surface of the Trans Golgi Network. The lysosomal proteins bind via these receptors, and are thereby separated from other secretory proteins. Subsequently, small transport vesicles containing the receptor-bound proteins are pinched off from the Trans Golgi Network and are targeted to their intracellular destination. See generally Kornfeld, Biochem. Soc. Trans. 18, 367-374 (1990).

There are over thirty lysosomal diseases, each resulting from a deficiency of a particular lysosomal protein, usually as a result of genetic mutation. See, e.g., Cotran et al., Robbins Pathologic Basis of Disease (4th ed. 1989) (incorporated by reference in its entirety for all purposes). The deficiency in the lysosomal protein usually results in harmful accumulation of a metabolite. For example, in Hurler's, Hunter's, Morquio's, and Sanfilippo's syndromes, there is an accumulation of mucopolysaccharides; in Tay-Sachs, Gaucher, Krabbe, Niemann-Pick, and Fabry syndromes, there is an accumulation of sphingolipids; and in fucosidosis and mannosidosis, there is an accumulation of fucose-containing sphingolipids and glycoprotein fragments, and of mannose-containing oligosaccharides, respectively.

Glycogen storage disease type II (GSD II; Pompe disease; acid maltase deficiency) is caused by deficiency of the lysosomal enzyme acid α-glucosidase (acid maltase). Two clinical forms are distinguished: early onset infantile and late onset, juvenile and adult. Infantile GSD II has its onset shortly after birth and presents with progressive muscular weakness and cardiac failure. This clinical variantis usually fatal within the first two years of life. Symptoms in the late onset in adult and juvenile patients occur later in life, and only skeletal muscles are involved. The patients eventually die due to respiratory insufficiency. Patients may exceptionally survive for more than six decades. There is a good correlation between the severity of the disease and the residual acid α-glucosidase activity, the activity being 10-20% of normal in late onset and less than 2% in early onset forms of the disease (see Hirschhorn, The Metabolic and Molecular Bases of Inherited Disease (Scriver et al., eds., 7th ed., McGraw-Hill, 1995), pp. 2443-2464).

Since the discovery of lysosomal enzyme deficiencies as the primary cause of lysosomal storage diseases (see, e.g., Hers, Biochem. J. 86, 11-16 (1963)), attempts have been made to treat patients having lysosomal storage diseases by (intravenous) administration of the missing enzyme, i.e., enzyme therapy. These experiments with enzyme replacement therapy for Pompe's disease were not successful. Either non-human α-glucosidase from Aspergillus niger, giving immunological reactions, or a form of the enzyme that is not efficiently taken up by cells (the low uptake form, mature enzyme from human placenta; see below) was used. Moreover, both the duration of treatment, and/or the amount of enzyme administered were insufficient (3-5). Production of lysosomal enzymes from natural sources such as human urine and bovine testis is in theory possible, but gives low yields, and the enzyme purified is not necessarily in a form that can be taken up by tissues of a recipient patient.

Notwithstanding the above uncertainties and difficulties, the invention provides methods of treating patients for Pompe's disease using human acid alpha glucosidase.

SUMMARY OF THE CLAIMED INVENTION

In one aspect, the invention provides methods of treating a patient with Pompe's disease. Such methods entail administering to the patient a therapeutically effective amount of human acid alpha glucosidase. The dosage is preferably at least 10 mg/kg body weight per week. In some methods, the dosage is at least 60 mg/kg body weight per week or at least 120 mg/kg body weight per week. In some methods, such dosages are administered on a single occasion per week and in other methods on three occasions per week. In some methods, the treatment is containued for ate least 24 weeks. Adminstration is preferably intravenous. The human acid alpha glucosidase is preferably obtained in the milk of a nonhuman transgenic mammal, and is preferably predominatly in a 110 kD form.

The methods can be used for treating patients with infantile, juvenile or adult Pompe's disease. In some methods of treating infantile Pompe's disease efficacy is indicated by a patient surviving to be at least one year old.

In some methods, levels of human acid alpha glucosidase are monitored in the recuouebt patient. Optionally, a second dosage of human acid alpha glucosidase can be administered if the level of alpha-glucosidase falls below a threshold value in the patient.

In some emthods, the human alpha glucosidase is administered intravenously and the rate of administration increases during the period of administration. In some methods, the rate of administration increases by at least a factor of ten during the period of administration. In some methods, the rate of administration increases by at least a factor of ten within a period of five hours. In some methods, the patient is administered a series of at least four dosages, each dosage at a higher strength than the previous dosage. In some methods, the dosages are a first dosage of 0.03-3 mg/kg/hr, a second dosage of 0.3-12 mg/kg/hr, a third dosage of 1-30 mg/kg/hr and a fourth dosage of 2-60 mg/kg/hr. In some

methods, the dosages are a first dosage of 0.1-1 mg/kg/hr, a second dosage of 1-4 mg/kg/hr, a third dosage of 3-10 mg/kg/hr and a fourth dosage of 6-20 mg/kg/hr. In some methods, the dosages are a first dosage of 0.25-4 mg/kg/hr, a second dosage of 0.9-1.4 mg/kg/hr, a third dosage of 3.6-5.7 mg/kg/hr and a fourth dosage of 7.2-11.3 mg/kg/hr. In some methods, the dosages are a first dosage of 0.3 mg/kg/hr, a second dosage of 1 mg/kg/hr, a third dosage of 4 mg/kg/hr and a fourth dosage of 12 mg/kg/hr. In some methods, the first, second, third and fourth dosages are each administered for periods of 15 min to 8 hours.

In some methods, the first, second, third and fourth dosages are administered for periods of 1 hr, 1 hr, 0.5 hr and 3 hr respectively.

In another aspect, the invention provides a pharmaceutical composition comprising human acid alpha glucosidase, human serum albumin, and a sugar in a physiologically acceptable buffer in sterile form. Some such compositions comprise human acid alpha glucosidase, human serum albumin, and glucose in sodium phosphate buffer. Some compositions comprise alpha glucosidase, mannitol and sucrose in an aqueous solution. In some compositions, the sugar comprises mannitol and sucrose and the concentration of mannitol is 1-3% w/w of the aqueous solution and the concentration of sucrose is 0.1 to 1% w/w of the aqueous solution. In some compositions, the concentration of mannitol is 2% w/w and the concentration of sucrose is 0.5% w/w.

The invention further provides a lyophilized composition produced by lyophilizing a pharmaceutical composition comprising human acid glucosidase, mannitol and sucrose in aqueous solution. Such a composition can be prepared by lyophilizing a first composition comprising human acid alpha-glucosidase, mannitol, sucrose and an aqueous solution to produce a second composition; and reconstituting the lyophilized composition in saline to produce a third composition. In some such compositions, the the human acid alpha-glucosidase is at 5 mg/ml in both the first and third composition, the mannitol is at 2 mg/ml in the first composition, the sucrose is at 0.5 mg/ml in the first composition, and the saline used in the reconstituting step is 0.9% w/w.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**: A transgene containing acid α-glucosidase cDNA. The αs1-casein exons are represented by open boxes; α-glucosidase cDNA is represented by a shaded box. The αS1-casein intron and flanking sequences are (SEQ ID NOS:2 and 3) represented by a thick line. A thin line represents the IgG acceptor site. The transcription initiation site is marked (1^{+}), the translation initiation site (ATG), the stop codon (TAG) and the polyadenylation site (pA).

FIG. **2** (panels A, B, C): Three transgenes containing acid α-glucosidase genomic DNA. Dark shaded areas are αS1 casein sequences, open boxes represent acids α-glucosidase exons, and the thin line between the open boxes represents α-glucosidase introns. Other symbols are the same as in FIG. **1**.

FIG. **3** (panels A, B, C): Construction of genomic transgenes. The α-glucosidase exons are represented by open boxes; the α-glucosidase introns and nontranslated sequences are indicated by thin lines. The pKUN vector sequences are represented by thick lines.

FIG. **4**. Detection of acid α-glucosidase in milk of transgenic mice by Western blotting.

DEFINITIONS

The term "substantial identity" or "substantial homology" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 65 percent sequence identity, preferably at least 80 or 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

The term "substantially pure" or "isolated" means an object species has been identified and separated and/or recovered from a component of its natural environment. Usually, the object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 to 90 percent by weight of all macromolecular species present in the composition. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of derivatives of a single macromolecular species.

A DNA segment is operably linked when placed into a functional relationship with another DNA segment. For example, DNA for a signal sequence is operably linked to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it stimulates the transcription of the sequence. Generally, DNA sequences that are operably linked are contiguous, and in the case of a signal sequence both contiguous and in reading phase. However, enhancers need not be contiguous with the coding sequences whose transcription they control. Linking is accomplished by ligation at convenient restriction sites or at adapters or linkers inserted in lieu thereof.

An exogenous DNA segment is one foreign to the cell, or homologous to a DNA segment of the cell but in an unnatural position in the host cell genome. Exogenous DNA segments are expressed to yield exogenous polypeptides.

In a transgenic mammal, all, or substantially all, of the germline and somatic cells contain a transgene introduced into the mammal or an ancestor of the mammal at an early embryonic stage.

DETAILED DESCRIPTION

The invention provides transgenic nonhuman mammals secreting a lysosomal protein into their milk. Secretion is achieved by incorporation of a transgene encoding a lysosomal protein and regulatory sequences capable of targeting expression of the gene to the mammary gland. The transgene is expressed, and the expression product posttranslationally modified within the mammary gland, and then secreted in milk. The posttranslational modification can include steps of glycosylation and phosphorylation to produce a mannose-6 phosphate containing lysosomal protein.

A. Lysosomal Genes

The invention provides transgenic nonhuman mammals expressing DNA segments containing any of the more than 30 known genes encoding lysosomal enzymes and other

types of lysosomal proteins, including α-glucosidase, α-L-iduronidase, iduronate-sulfate sulfatase, hexosaminidase A and B, ganglioside activator protein, arylsulfatase A and B, iduronate sulfatase, heparan N-sulfatase, galacto-ceramidase, α-galactosylceramidase A, sphingomyelinase, α-fucosidase, α-mannosidase, aspartylglycosamine amide hydrolase, acid lipase, N-acetyl-α-D-glucosamine-6-sulphate sulfatase, α- and β-galactosidase, β-glucuronidase, β-mannosidase, ceramidase, galacto-cere-brosidase, α-N-acetylgalactosaminidase, and protective protein and others. Transgenic mammals expressing allelic, cognate and induced variants of any of the known lysosomal protein gene sequences are also included. Such variants usually show substantial sequence identity at the amino acid level with known lysosomal protein genes. Such variants usually hybridize to a known gene under stringent conditions or crossreact with antibodies to a polypeptide encoded by one of the known genes.

DNA clones containing the genomic or cDNA sequences of many of the known genes encoding lysosomal proteins are available. (Scott et al., Am. J. Hum. Genet. 47, 802-807 (1990); Wilson et al., PNAS 87, 8531-8535 (1990): Stein et al., J. Biol. Chem. 264, 1252-1259 (1989); Ginns et al., Biochem. Biophys. Res. Comm. 123, 574-580 (1984); Hoefsloot et al., EMBO J. 7, 1697-1704 (1988); Hoefsloot et al., Biochem. J. 272, 473-479 (1990); Meyerowitz & Proia, PNAS 81, 5394-5398 (1984); Scriver et al., supra, part 12, pages 2427-2882 and references cited therein)) Other examples of genomic and cDNA sequences are available from GenBank. To the extent that additional cloned sequences of lysosomal genes are required, they may be obtained from genomic or cDNA libraries (preferably human) using known lysosomal protein DNA sequences or antibodies to known lysosomal proteins as probes.

B. Conformaion of Lysosomal Proteins

Recombinant lysosomal proteins are preferably processed to have the same or similar structure as naturally occurring lysosomal proteins. Lysosomal proteins are glycoproteins that are synthesized on ribosomes bound to the endoplasmic reticulum (RER). They enter this organelle co-translationally guided by an N-terminal signal peptide (Ng et al., Current Opinion in Cell Biology 6, 510-516 (1994)). The N-linked glycosylation process starts in the RER with the en bloc transfer of the high-mannose oligosaccharide precursor Glc3Man9GlcNAc2 from a dolichol carrier. Carbohydrate chain modification starts in the RER and continue in the Golgi apparatus with the removal of the three outermost glucose residues by glycosidases I and II. Phosphorylation is a two-step procedure in which first N-acetyl-gluco-samine-1-phosphate is coupled to select mannose groups by a lysosomal protein specific transferase, and second, the N-acetyl-gluco-samine is cleaved by a diesterase (Goldberg et al., Lysosomes: Their Role in Protein Breakdown (Academic Press Inc., London, 1987), pp. 163-191). Cleavage exposes mannose 6-phosphate as a recognition marker and ligand for the mannose 6-phosphate receptor mediating transport of most lysosomal proteins to the lysosomes (Kornfeld, Biochem. Soc. Trans. 18, 367-374 (1992)).

In addition to carbohydrate chain modification, most lysosomal proteins undergo proteolytic processing, in which the first event is removal of the signal peptide. The signal peptide of most lysosomal proteins is cleaved after translocation by signal peptidase after which the proteins become soluble. There is suggestive evidence that the signal peptide of acid α-glucosidase is cleaved after the enzyme has left the RER, but before it has entered the lysosome or the secretory pathway (Wisselaar et al., J. Biol. Chem. 268, 2223-2231 (1993)). The proteolytic processing of acid at-glucosidase is complex and involves a series of steps in addition to cleavage of the signal peptide taking place at various subcellular locations. Polypeptides are cleaved off at both the N and C terminal ends, whereby the specific catalytic activity is increased. The main species recognized are a 110/100 kD precursor, a 95 kD intermediate and 76 kD and 70 kD mature forms. (Hasilik et al., J. Biol. Chem. 255, 4937-4945 (1980); Oude Elferink et al., Eur. J. Biochem. 139, 489-495 (1984); Reuser et al., J. Biol. Chem. 260, 8336-8341 (1985); Hoefsloot et al., EMBO J. 7, 1697-1704 (1988)). The post translational processing of natural human acid α-glucosidase and of recombinant forms of human acid α-glucosidase as expressed in cultured mammalian cells like COS cells, BHK cells and CHO cells is similar (Hoefsloot et al., (1990) supra; Wisselaar et al., (1993) supra.

Authentic processing to generate lysosomal proteins phosphorylated at the 6' position of the mannose group can be tested by measuring uptake of a substrate by cells bearing a receptor for mannose 6-phosphate. Correctly modified substrates are taken up faster than unmodified substrates, and in a manner whereby uptake of the modified substrate can be competitively inhibited by addition of mannose 6-phosphate.

C. Transgene Design

Transgenes are designed to target expression of a recombinant lysosomal protein to the mammary gland of a transgenic nonhuman mammal harboring the transgene. The basic approach entails operably linking an exogenous DNA segment encoding the protein with a signal sequence, a promoter and an enhancer. The DNA segment can be genomic, minigene (genomic with one or more introns omitted), cDNA, a YAC fragment, a chimera of two different lysosomal protein genes, or a hybrid of any of these. Inclusion of genomic sequences generally leads to higher levels of expression. Very high levels of expression might overload the capacity of the mammary gland to perform posttranslation modifications, and secretion of lysosomal proteins. However, the data presented below indicate that substantial posttranslational modification occurs including the formation of mannose 6-phosphate groups, notwithstanding a high expression level in the mg/ml range. Substantial modification means that at least about 10, 25, 50, 75 or 90% of secreted molecules bear at least one mannose 6-phosphate group. Thus, genomic constructs or hybrid cDNA-genomic constructs are generally preferred.

In genomic constructs, it is not necessary to retain all intronic sequences. For example, some intronic sequences can be removed to obtain a smaller transgene facilitating DNA manipulations and subsequent microinjection. See Archibald et al., WO 90/05188 (incorporated by reference in its entirety for all purposes). Removal of some introns is also useful in some instances to reduce expression levels and thereby ensure that posttranslational modification is substantially complete. In other instances excluding an intron such as intron one from the genomic sequence of acid α-glucosidase leads to a higher expression of the mature enzyme. It is also possible to delete some or all of noncoding exons. In some transgenes, selected nucleotides in lysosomal protein encoding sequences are mutated to remove proteolytic cleavage sites.

Because the intended use of lysosomal proteins produced by transgenic mammals is usually administration to humans, the species from which the DNA segment encoding a lysosomal protein sequence is obtained is preferably human. Analogously if the intended use were in veterinary therapy

(e.g., on a horse, dog or cat), it is preferable that the DNA segment be from the same species.

The promoter and enhancer are from a gene that is exclusively or at least preferentially expressed in the mammary gland (i.e., a mammary-gland specific gene). Preferred genes as a source of promoter and enhancer include β-casein, κ-casein, αS1-casein, αS2-casein, β-lactoglobulin, whey acid protein, and α-lactalbumin. The promoter and enhancer are usually but not always obtained from the same mammary-gland specific gene. This gene is sometimes but not necessarily from the same species of mammal as the mammal into which the transgene is to be expressed. Expression regulation sequences from other species such as those from human genes can also be used. The signal sequence must be capable of directing the secretion of the lysosomal protein from the mammary gland. Suitable signal sequences can be derived from mammalian genes encoding a secreted protein. Surprisingly, the natural signal sequences of lysosomal proteins are suitable, notwithstanding that these proteins are normally not secreted but targeted to an intracellular organelle. In addition to such signal sequences, preferred sources of signal sequences are the signal sequence from the same gene as the promoter and enhancer are obtained. Optionally, additional regulatory sequences are included in the transgene to optimize expression levels. Such sequences include 5' flanking regions, 5' transcribed but untranslated regions, intronic sequences, 3' transcribed but untranslated regions, polyadenylation sites, and 3' flanking regions. Such sequences are usually obtained either from the mammary-gland specific gene from which the promoter and enhancer are obtained or from the lysosomal protein gene being expressed. Inclusion of such sequences produces a genetic milieu simulating that of an authentic mammary gland specific gene and/or that of an authentic lysosomal protein gene. This genetic milieu results in some cases (e.g., bovine αS1-casein) in higher expression of the transcribed gene. Alternatively, 3' flanking regions and untranslated regions are obtained from other heterologous genes such as the β-globin gene or viral genes. The inclusion of 3' and 5' untranslated regions from a lysosomal protein gene, or other heterologous gene can also increase the stability of the transcript.

In some embodiments, about 0.5, 1, 5, 10, 15, 20 or 30 kb of 5' flanking sequence is included from a mammary specific gene in combination with about 1, 5, 10, 15, 20 or 30 kb or 3' flanking sequence from the lysosomal protein gene being expressed. If the protein is expressed from a cDNA sequence, it is advantageous to include an intronic sequence between the promoter and the coding sequence. The intronic sequence is preferably a hybrid sequence formed from a 5' portion from an intervening sequence from the first intron of the mammary gland specific region from which the promoter is obtained and a 3' portion from an intervening sequence of an IgG intervening sequence or lysosomal protein gene. See DeBoer et al., WO 91/08216 (incorporated by reference in its entirety for all purposes).

A preferred transgene for expressing a lysosomal protein comprises a cDNA-genomic hybrid lysosomal protein gene linked 5' to a casein promoter and enhancer. The hybrid gene includes the signal sequence, coding region, and a 3' flanking region from the lysosomal protein gene. Optionally, the cDNA segment includes an intronic sequence between the 5' casein and untranslated region of the gene encoding the lysosomal protein. Of course, corresponding cDNA and genomic segments can also be fused at other locations within the gene provided a contiguous protein can be expressed from the resulting fusion.

Other preferred transgenes have a genomic lysosomal protein segment linked 5' to casein regulatory sequences. The genomic segment is usually contiguous from the 5' untranslated region to the 3' flanking region of the gene. Thus, the genomic segment includes a portion of the lysosomal protein 5' untranslated sequence, the signal sequence, alternating introns and coding exons, a 3' untranslated region, and a 3' flanking region. The genomic segment is linked via the 5' untranslated region to a casein fragment comprising a promoter and enhancer and usually a 5' untranslated region.

DNA sequence information is available for all of the mammary gland specific genes listed above, in at least one, and often several organisms. See, e.g., Richards et al., J. Biol. Chem. 256, 526-532 (1981) (α-lactalbumin rat); Campbell et al., Nucleic Acids Res. 12, 8685-8697 (1984) (rat WAP); Jones et al., J. Biol. Chem. 260, 7042-7050 (1985)) (rat β-casein); Yu-Lee & Rosen, J. Biol. Chem. 258, 10794-10804 (1983) (rat γ-casein)); Hall, Biochem. J. 242, 735-742 (1987) (α-lactalbumin human); Stewart, Nucleic Acids Res. 12, 389 (1984) (bovine αs1 and κ casein cDNAs); Gorodetsky et al., Gene 66, 87-96 (1988) (bovine β casein); Alexander et al., Eur. J. Biochem. 178, 395-401 (1988) (bovine κ casein); Brignon et al., FEBS Lett. 188, 48-55 (1977) (bovine αS2 casein); Jamieson et al., Gene 61, 85-90 (1987), Ivanov et al., Biol. Chem. Hoppe-Seyler 369, 425-429 (1988), Alexander et al., Nucleic Acids Res. 17, 6739 (1989) (bovine β lactoglobulin); Vilotte et al., Biochimie 69, 609-620 (1987) (bovine α-lactalbumin) (incorporated by reference in their entirety for all purposes). The structure and function of the various milk protein genes are reviewed by Mercier & Vilotte, J. Dairy Sci. 76, 3079-3098 (1993) (incorporated by reference in its entirety for all purposes). To the extent that additional sequence data might be required, sequences flanking the regions already obtained could be readily cloned using the existing sequences as probes. Mammary-gland specific regulatory sequences from different organisms are likewise obtained by screening libraries from such organisms using known cognate nucleotide sequences, or antibodies to cognate proteins as probes.

General strategies and exemplary transgenes employing αS1-casein regulatory sequences for targeting the expression of a recombinant protein to the mammary gland are described in more detail in DeBoer et al., WO 91/08216 and WO 93/25567 (incorporated by reference in their entirety for all purposes). Examples of transgenes employing regulatory sequences from other mammary gland specific genes have also been described. See, e.g., Simon et al., Bio/Technology 6, 179-183 (1988) and WO88/00239 (1988) (β-lactoglobulin regulatory sequence for expression in sheep); Rosen, EP 279,582 and Lee et al., Nucleic Acids Res. 16, 1027-1041 (1988) (β-casein regulatory sequence for expression in mice); Gordon, Biotechnology 5, 1183 (1987) (WAP regulatory sequence for expression in mice); WO 88/01648 (1988) and Eur. J. Biochem. 186, 43-48 (1989) (α-lactalbumin regulatory sequence for expression in mice) (incorporated by reference in their entirety for all purposes).

The expression of lysosomal proteins in the milk from transgenes can be influenced by co-expression or functional inactivation (i.e., knock-out) of genes involved in post translational modification and targeting of the lysosomal proteins. The data in the Examples indicate that surprisingly mammary glands already express modifying enzymes at sufficient quantities to obtain assembly and secretion of mannose 6-phosphate containing proteins at high levels. However, in some transgenic mammals expressing these proteins at high levels, it is sometimes preferable to supple-

ment endogenous levels of processing enzymes with additional enzyme resulting from transgene expression. Such transgenes are constructed employing similar principles to those discussed above with the processing enzyme coding sequence replacing the lysosomal protein coding sequence in the transgene. It is not generally necessary that posttranslational processing enzymes be secreted. Thus, the secretion signal sequence linked to the lysosomal protein coding sequence is replaced with a signal sequence that targets the processing enzyme to the endoplasmic reticulum without secretion. For example, the signal sequences naturally associated with these enzymes are suitable.

D. Transgenesis

The transgenes described above are introduced into nonhuman mammals. Most nonhuman mammals, including rodents such as mice and rats, rabbits, ovines such as sheep and goats, porcines such as pigs, and bovines such as cattle and buffalo, are suitable. Bovines offer an advantage of large yields of milk, whereas mice offer advantages of ease of transgenesis and breeding. Rabbits offer a compromise of these advantages. A rabbit can yield 100 ml milk per day with a protein content of about 14% (see Buhler et al., Biotechnology 8, 140 (1990)) (incorporated by reference in its entirety for all purposes), and yet can be manipulated and bred using the same principles and with similar facility as mice. Nonviviparous mammals such as a spiny anteater or duckbill platypus are typically not employed.

In some methods of transgenesis, transgenes are introduced into the pronuclei of fertilized oocytes. For some animals, such as mice and rabbits, fertilization is performed in vivo and fertilized ova are surgically removed. In other animals, particularly bovines, it is preferable to remove ova from live or slaughterhouse animals and fertilize the ova in vitro. See DeBoer et al., WO 91/08216. In vitro fertilization permits a transgene to be introduced into substantially synchronous cells at an optimal phase of the cell cycle for integration (not later than S-phase). Transgenes are usually introduced by microinjection. See U.S. Pat. No. 4,873,292. Fertilized oocytes are then cultured in vitro until a pre-implantation embryo is obtained containing about 16-150 cells. The 16-32 cell stage of an embryo is described as a morula. Pre-implantation embryos containing more than 32 cells are termed blastocysts. These embryos show the development of a blastocoele cavity, typically at the 64 cell stage. Methods for culturing fertilized oocytes to the pre-implantation stage are described by Gordon et al., Methods Enzymol. 101, 414 (1984); Hogan et al., Manipulation of the Mouse Embryo: A Laboratory Manual, C.S.H.L. N.Y. (1986) (mouse embryo); and Hammer et al., Nature 315, 680 (1985) (rabbit and porcine embryos); Gandolfi et al. J. Reprod. Fert. 81, 23-28 (1987); Rexroad et al., J. Anim. Sci. 66, 947-953 (1988) (ovine embryos) and Eyestone et al. J. Reprod. Fert. 85, 715-720 (1989); Camous et al., J. Reprod. Fert. 72, 779-785 (1984); and Heyman et al. Theriogenology 27, 5968 (1987) (bovine embryos) (incorporated by reference in their entirety for all purposes). Sometimes pre-implantation embryos are stored frozen for a period pending implantation. Pre-implantation embryos are transferred to the oviduct of a pseudopregnant female resulting in the birth of a transgenic or chimeric animal depending upon the stage of development when the transgene is integrated. Chimeric mammals can be bred to form true germline transgenic animals.

Alternatively, transgenes can be introduced into embryonic stem cells (ES). These cells are obtained from pre-implantation embryos cultured in vitro. Bradley et al., Nature 309, 255-258 (1984) (incorporated by reference in its entirety for all purposes). Transgenes can be introduced into such cells by electroporation or microinjection. Transformed ES cells are combined with blastocysts from a non-human animal. The ES cells colonize the embryo and in some embryos form the germline of the resulting chimeric animal. See Jaenisch, Science, 240, 1468-1474 (1988) (incorporated by reference in its entirety for all purposes). Alternatively, ES cells can be used as a source of nuclei for transplantation into an enucleated fertilized oocyte giving rise to a transgenic mammal.

For production of transgenic animals containing two or more transgenes, the transgenes can be introduced simultaneously using the same procedure as for a single transgene. Alternatively, the transgenes can be initially introduced into separate animals and then combined into the same genome by breeding the animals. Alternatively, a first transgenic animal is produced containing one of the transgenes. A second transgene is then introduced into fertilized ova or embryonic stem cells from that animal. In some embodiments, transgenes whose length would otherwise exceed about 50 kb, are constructed as overlapping fragments. Such overlapping fragments are introduced into a fertilized oocyte or embryonic stem cell simultaneously and undergo homologous recombination in vivo. See Kay et al., WO 92/03917 (incorporated by reference in its entirety for all purposes).

E. Characteristics of Transgenic Mammals

Transgenic mammals of the invention incorporate at least one transgene in their genome as described above. The transgene targets expression of a DNA segment encoding a lysosomal protein at least predominantly to the mammary gland. Surprisingly, the mammary glands are capable of expressing proteins required for authentic posttranslation processing including steps of oligosaccharide addition and phosphorylation. Processing by enzymes in the mammary gland results in phosphorylation of the 6' position of mannose groups.

Lysosomal proteins can be secreted at high levels of at least 10, 50, 100, 500, 1000, 2000, 5000 or 10,000 μg/ml. Surprisingly, the transgenic mammals of the invention exhibit substantially normal health. Secondary expression of lysosomal proteins in tissues other than the mammary gland does not occur to an extent sufficient to cause deleterious effects. Moreover, exogenous lysosomal protein produced in the mammary gland is secreted with sufficient efficiency that no significant problem is presented by deposits clogging the secretory apparatus.

The age at which transgenic mammals can begin producing milk, of course, varies with the nature of the animal. For transgenic bovines, the age is about two-and-a-half years naturally or six months with hormonal stimulation, whereas for transgenic mice the age is about 5-6 weeks. Of course, only the female members of a species are useful for producing milk. However, transgenic males are also of value for breeding female descendants. The sperm from transgenic males can be stored frozen for subsequent in vitro fertilization and generation of female offspring.

F. Recovery of Proteins from Milk

Transgenic adult female mammals produce milk containing high concentrations of exogenous lysosomal protein. The protein can be purified from milk, if desired, by virtue of its distinguishing physical and chemical properties, and standard purification procedures such as precipitation, ion exchange, molecular exclusion or affinity chromatography. See generally Scopes, Protein Purification (Springer-Verlag, N.Y., 1982).

Purification of human acid α-glucosidase from milk can be carried out by defatting of the transgenic milk by cen-

trifugation and removal of the fat, followed by removal of caseins by high speed centrifugation followed by dead-end filtration (i.e., dead-end filtration by using successively declining filter sizes) or cross-flow filtration, or; removal of caseins directly by cross-flow filtration. Human acid α-glucosidase is purified by chromatography, including Q Sepharose FF (or other anion-exchange matrix), hydrophobic interaction chromatography (HIC), metal-chelating Sepharose, or lectins coupled to Sepharose (or other matrices).

Q Sepharose Fast Flow chromatography may be used to purify human acid α-glucosidase present in filtered whey or whey fraction as follows: a Q Sepharose Fast Flow (QFF; Pharmacia) chromatography (Pharmacia XK-50 column, 15 cm bed height; 250 cm/hr flow rate) the column was equilibrated in 20 mM sodiumphosphate buffer, pH 7.0 (buffer A); the S/D-incubated whey fraction (about 500 to 600 ml) is loaded and the column is washed with 4-6 column volumes (cv) of buffer A (20 mM sodium phosphate buffer, pH 7.0). The human acid α-glucosidase fraction is eluted from the Q FF column with 2-3 cv buffer A, containing 100 mM NaCl.

The Q FF Sepharose human acid α-glucosidase containing fraction can be further purified using Phenyl Sepharose High Performance chromatography. For example, 1 vol. of 1 M ammonium sulphate is added to the Q FF Sepharose human acid α-glucosidase eluate while stirring continuously. Phenyl HP (Pharmacia) column chromatography (Pharmacia XK-50 column, 15 cm bed height; 150 cm/hr flow rate) is then done at room temperature by equilibrating the column in 0.5 M ammonium sulphate, 50 mM sodiumphosphate buffer pH 6.0 (buffer C), loading the 0.5 M ammoniumsulphate-incubated human acid α-glucosidase eluate (from Q FF Sepharose), washing the column with 2-4 cv of buffer C, and eluting the human acid α-glucosidase was eluted from the Phenyl HP column with 3-5 cv buffer D (50 mM sodiumphosphate buffer at pH 6.0). Alternative methods and additional methods for further purifying human acid α-glucosidase will be apparent to those of skill. For example, see United Kingdom patent application 998 07464.4 (incorporated by reference in its entirety for all purposes).

G. Uses of Recombinant Lysosomal Proteins

The recombinant lysosomal proteins produced according to the invention find use in enzyme replacement therapeutic procedures. A patient having a genetic or other deficiency resulting in an insufficiency of functional lysosomal enzyme can be treated by administering exogenous enzyme to the patient. Patients in need of such treatment can be identified from symptoms (e.g., Hurler's syndrome symptoms include Dwarfism, corneal clouding, hepatosplenomegaly, valvular lesions, coronary artery lesions, skeletal deformities, joint stiffness and progressive mental retardation). Alternatively, or additionally, patients can be diagnosed from biochemical analysis of a tissue sample to reveal excessive accumulation of a characteristic metabolite processed by a particular lysosomal enzyme or by enzyme assay using an artificial or natural substrate to reveal deficiency of a particular lysosomal enzyme activity. For most diseases, diagnosis can be made by measuring the particular enzyme deficiency or by DNA analysis before occurrence of symptoms or excessive accumulation of metabolites (Scriver et al., supra, chapters on lysosomal storage disorders). All of the lysosomal storage diseases are hereditary. Thus, in offspring from families known to have members suffering from lysosomal diseases, it is sometimes advisable to commence prophylactic treatment even before a definitive diagnosis can be made.

Pharmaceutical Compositions

In some methods, lysosomal enzymes are administered in purified form together with a pharmaceutical carrier as a pharmaceutical composition. The preferred form depends on the intended mode of administration and therapeutic application. The pharmaceutical carrier can be any compatible, nontoxic substance suitable to deliver the polypeptides to the patient. Sterile water, alcohol, fats, waxes, and inert solids may be used as the carrier. Pharmaceutically-acceptable adjuvants, buffering agents, dispersing agents, and the like, may also be incorporated into the pharmaceutical compositions.

The concentration of the enzyme in the pharmaceutical composition can vary widely, i.e., from less than about 0.1% by weight, usually being at least about 1% by weight to as much as 20% by weight or more.

For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. Active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

A typical composition for intravenous infusion could be made up to contain 100 to 500 ml of sterile 0.9% NaCl or 5% glucose optionally supplemented with a 20% albumin solution and 100 to 500 mg of an enzyme. A typical pharmaceutical compositions for intramuscular injection would be made up to contain, for example, 1 ml of sterile buffered water and 1 to 10 mg of the purified alpha glucosidase of the present invention. Methods for preparing parenterally administrable compositions are well known in the art and described in more detail in various sources, including, for example, Remington's Pharmaceutical Science (15th ed., Mack Publishing, Easton, Pa., 1980) (incorporated by reference in its entirety for all purposes).

AGLU can be formulated in 10 mM sodium phosphate buffer pH 7.0. Small amounts of ammonium sulphate are optionally present (<10 mM). The enzyme is typically kept frozen at about −70° C., and thawed before use. Alternatively, the enzyme may be stored cold (e.g., at about 4° C. to 8° C.) in solution. In some embodiments, AGLU solutions comprise a buffer (e.g., sodium phosphate, potassium phosphate or other physiologically acceptable buffers), a simple carbohydrate (e.g., sucrose, glucose, maltose, mannitol or the like), proteins (e.g., human serum albumin), and/or surfactants (e.g., polysorbate 80 (Tween-80), cremophore-EL, cremophore-R, labrofil, and the like).

AGLU can also be stored in lyophilized form. For lyophilization, AGLU can be formulated in a solution containing mannitol, and sucrose in a phosphate buffer. The concentration of sucrose should be sufficient to prevent

aggregation of AGLU on reconstitution. The concentration of mannitol should be sufficient to significantly reduce the time otherwise needed for lyophilization. The concentrations of mannitol and sucrose should, however, be insufficient to cause unacceptable hypertonicity on reconstitution. Concentrations of mannitol and sucrose of 1-3 mg/ml and 0.1-1.0 mg/ml respectively are suitable. Preferred concentrations are 2 mg/ml mannitol and 0.5 mg/ml sucrose. AGLU is preferably at 5 mg/ml before lyophilization and after reconstitution. Saline preferably at 0.9% is a preferred solution for reconstitution.

For AGLU purified from rabbit milk, a small amount of impurities (e.g., up to about 5%) can be tolerated. Possible impurities may be present in the form of rabbit whey proteins. Other possible impurities are structural analogues (e.g., oligomers and aggregates) and truncations of AGLU. Current batches indicate that the AGLU produced in transgenic rabbits is >95% pure. The largest impurities are rabbit whey proteins, although on gel electrophoresis, AGLU bands of differing molecular weights are also seen.

Infusion solutions should be prepared aseptically in a laminar air flow hood. The appropriate amount of AGLU should be removed from the freezer and thawed at room temperature. Infusion solutions can be prepared in glass infusion bottles by mixing the appropriate amount of AGLU finished product solution with an adequate amount of a solution containing human serum albumin (HSA) and glucose. The final concentrations can be 1% HSA and 4% glucose for 25-200 mg doses and 1% HSA and 4% glucose for 400-800 mg doses. HSA and AGLU can be filtered with a 0.2 μm syringe filter before transfer into the infusion bottle containing 5% glucose. Alternatively, AGLU can be reconstituted in saline solution, preferably 0.9% for infusion. Solutions of AGLU for infusion have been shown to be stable for up to 7 hours at room temperature. Therefore the AGLU solution is preferably infused within seven hours of preparation.

Therapeutic Methods

The present invention provides effective methods of treating Pompe's disease. These methods are premised in part on the availability of large amounts of human acid alpha glucosidase in a form that is catalytically active and in a form that can be taken up by tissues, particularly, liver, heart and muscle (e.g., smooth muscle, striated muscle, and cardiac muscle), of a patient being treated. Such human acid alpha-glucosidase is provided from e.g., the transgenic animals described in the Examples. The alpha-glucosidase is preferably predominantly (i.e., >50%) in the precursor form of about 100-110 kD. (The apparent molecular weight or relative mobility of the 100-110 kD precursor may vary somewhat depending on the method of analysis used, but is typically within the range 95 kD and 120 kD.) Given the successful results with human acid alpha-glucosidase in the transgenic animals discussed in the Examples, it is possible that other sources of human alpha-glucosidase, such as resulting from cellular expression systems, can also be used. For example, an alternative way to produce human acid α-glucosidase is to transfect the acid α-glucosidase gene into a stable eukaryotic cell line (e.g., CHO) as a cDNA or genomic construct operably linked to a suitable promoter. However, it is more laborious to produce the large amounts of human acid alpha glucosidase needed for clinical therapy by such an approach.

The pharmaceutical compositions of the present invention are usually administered intravenously. Intradermal, intramuscular or oral administration is also possible in some circumstances. The compositions can be administered for prophylactic treatment of individuals suffering from, or at risk of, a lysosomal enzyme deficiency disease. For therapeutic applications, the pharmaceutical compositions are administered to a patient suffering from established disease in an amount sufficient to reduce the concentration of accumulated metabolite and/or prevent or arrest further accumulation of metabolite. For individuals at risk of lysosomal enzyme deficiency disease, the pharmaceutical compositions are administered prophylactically in an amount sufficient to either prevent or inhibit accumulation of metabolite. An amount adequate to accomplish this is defined as a "therapeutically-" or "prophylactically-effective dose." Such effective dosages will depend on the severity of the condition and on the general state of the patient's health.

In the present methods, human acid alpha glucosidase is usually administered at a dosage of 10 mg/kg patient body weight or more per week to a patient. Often dosages are greater than 10 mg/kg per week. Dosages regimes can range from 10 mg/kg per week to at least 1000 mg/kg per week. Typically dosage regimes are 10 mg/kg per week, 15 mg/kg per week, 20 mg/kg per week, 25 mg/kg per week, 30 mg/kg per week, 35 mg/kg per week, 40 mg/kg week, 45 mg/kg per week, 60 mg/kg week, 80 mg/kg per week and 120 mg/kg per week. In preferred regimes 10 mg/kg, 15 mg/kg, 20 mg/kg, 30 mg/kg or 40 mg/kg is administered once, twice or three times weekly. Treatment is typically continued for at least 4 weeks, sometimes 24 weeks, and sometimes for the life of the patient. Treatment is preferably administered i.v. Optionally, levels of human alpha-glucosidase are monitored following treatment (e.g., in the plasma or muscle) and a further dosage is administered when detected levels fall substantially below (e.g., less than 20%) of values in normal persons.

In some methods, human acid alpha glucosidase is administered at an initially "high" dose (i.e., a "loading dose"), followed by administration of a lower doses (i.e., a "maintenance dose"). An example of a loading dose is at least about 40 mg/kg patient body weight 1 to 3 times per week (e.g., for 1, 2, or 3 weeks). An example of a maintenance dose is at least about 5 to at least about 10 mg/kg patient body weight per week, or more, such as 20 mg/kg per week, 30 mg/kg per week, 40 mg/kg week.

In some methods, a dosage is administered at increasing rate during the dosage period. Such can be achieved by increasing the rate of flow intravenous infusion or by using a gradient of increasing concentration of alpha-glucosidase administered at constant rate. Administration in this manner reduces the risk of immunogenic reaction. In some dosages, the rate of administration measured in units of alpha glucosidase per unit time increases by at least a factor of ten. Typically, the intravenous infusion occurs over a period of several hours (e.g., 1-10 hours and preferably 2-8 hours, more preferably 3-6 hours), and the rate of infusion is increased at intervals during the period of administration.

Suitable dosages (all in mg/kg/hr) for infusion at increasing rates are shown in table 1 below. The first column of the table indicates periods of time in the dosing schedule. For example, the reference to 0-1 hr refers to the first hour of the dosing. The fifth column of the table shows the range of doses than can be used at each time period. The fourth column shows a narrower included range of preferred dosages. The third column indicates upper and lower values of dosages administered in an exemplary clinical trial. The second column shows particularly preferred dosages, these representing the mean of the range shown in the third column of table 1.

TABLE 1

Time	Mean Doses (I)	Lower & Upper Values	Preferred Range	Range
0-1 hr:	0.3 mg/kg/hr	0.25-0.4	0.1-1	0.03-3
1-2 hr:	1 mg/kg/hr	0.9-1.4	1-4	0.3-12
2-2.5 hr:	4 mg/kg/hr	3.6-5.7	3-10	1-30
2.5-5.6 hr.	12 mg/kg/hr	7.2-11.3	6-20	2-60

The methods are effective on patients with both early onset (infantile) and late onset (juvenile and adult) Pompe's disease. In patients with the infantile form of Pompe's disease symptoms become apparent within the first 4 months of life. Mostly, poor motor development and failure to thrive are noticed first. On clinical examination, there is generalized hypotonia with muscle wasting, increased respiration rate with sternal retractions, moderate enlargement of the liver, and protrusion of the tongue. Ultrasound examination of the heart shows a progressive hypertrophic cardiomyopathy, eventually leading to insufficient cardiac output. The ECG is characterized by marked left axis deviation, a short PR interval, large QRS complexes, inverted T waves and ST depression. The disease shows a rapidly progressive course leading to cardiorespiratory failure within the first year of life. On histological examination at autopsy lysosomal glycogen storage is observed in various tissues, and is most pronounced in heart and skeletal muscle. Treatment with human acid alpha glucosidase in the present methods results in a prolongation of life of such patients (e.g., greater than 1, 2, 5 years up to a normal lifespan). Treatment can also result in elimination or reduction of clinical and biochemical characteristics of Pompe's disease as discussed above. Treatment is administered soon after birth, or antenatally if the parents are known to bear variant alpha glucosidase alleles placing their progeny at risk.

Patients with the late onset adult form of Pompe's disease may not experience symptoms within the first two decades of life. In this clinical subtype, predominantly skeletal muscles are involved with predilection of those of the limb girdle, the trunk and the diaphragm. Difficulty in climbing stairs is often the initial complaint. The respiratory impairment varies considerably. It can dominate the clinical picture, or it is not experienced by the patient until late in life. Most such patients die because of respiratory insufficiency. In patients with the juvenile subtype, symptoms usually become apparent in the first decade of life. As in adult Pompe's disease, skeletal muscle weakness is the major problem; cardiomegaly, hepatomegaly, and macroglossia can be seen, but are rare. In many cases, nightly ventilatory support is ultimately needed. Pulmonary infections in combination with wasting of the respiratory muscles are life threatening and mostly become fatal before the third decade. Treatment with the present methods prolongs the life of patients with late onset juvenile or adult Pompe's disease up to a normal life span. Treatment also eliminates or significantly reduces clinical and biochemical symptoms of disease.

Other Uses

Lysosomal proteins produced in the milk of transgenic animals have a number of other uses. For example, α-glucosidase, in common with other α-amylases, is an important tool in production of starch, beer and pharmaceuticals. See. Vihinen & Mantsala. Crit. Rev. Biochem. Mol. Biol. 24, 329-401 (1989) (incorporated by reference in its entirety for all purpose). Lysosomal proteins are also useful for producing laboratory chemicals or food products. For example, acid α-glucosidase degrades 1,4 and 1,6 α-glucidic bonds and can be used for the degradation of various carbohydrates containing these bonds, such as maltose, isomaltose, starch and glycogen, to yield glucose. Acid α-glucosidase is also useful for administration to patients with an intestinal maltase or isomaltase deficiency. Symptoms otherwise resulting from the presence of undigested maltose are avoided. In such applications, the enzyme can be administered without prior fractionation from milk, as a food product derived from such milk (e.g., ice cream or cheese) or as a pharmaceutical composition. Purified recombinant lysosomal enzymes are also useful for inclusion as controls in diagnostic kits for assay of unknown quantities of such enzymes in tissue samples.

EXAMPLES

Example 1

Construction of Transgenes

(a) cDNA Construct

Construction of an expression vector containing cDNA encoding human acid α-glucosidase started with the plasmid p16,8hlf3 (see DeBoer et al. (1991) & (1993), supra) This plasmid includes bovine αS1-casein regulatory sequences. The lactoferrin cDNA insert of the parent plasmid was exchanged for the human acid α-glucosidase cDNA (Hoefsloot et al. EMBO J. 7, 1697-1704 (1988)) at the ClaI site and SalI site of the expression cassette as shown in FIG. **1**. To obtain the compatible restriction sites at the ends of the α-glucosidase cDNA fragment, plasmid pSHAG2 (id.) containing the complete cDNA encoding human α-glucosidase was digested with EcoRI and SphI and the 3.3 kb cDNA-fragment was subcloned in pKUN7ΔC a pKUN1 derivative (Konings et al., Gene 46, 269-276 (1986)), with a destroyed ClaI site within the vector nucleotide sequences and with a newly designed polylinker: HindIII ClaI EcoRI SphI XhoI EcoRI SfiI SfiI/SmaI NotI EcoRI*(*=destroyed site). From the resulting plasmid pagluCESX, the 3.3-kb cDNA-fragment could be excised by ClaI and XhoI. This fragment was inserted into the expression cassette shown in FIG. **1** at the ClaI site and XhoI-compatible SalI site. As a result, the expression plasmid p16,8αglu consists of the cDNA sequence encoding human acid α-glucosidase flanked by bovine αS1-casein sequences as shown in FIG. **1**. The 27.3-kb fragment containing the complete expression cassette can be excised by cleavage with NotI (see FIG. **1**).

(b) Genomic Constructs

Construct c8αgluex1 contains the human acid α-glucosidase gene (Hoefsloot et al., Biochem. J. 272, 493-497 (1990)): starting in exon 1 just downstream of its transcription initiation site (see FIG. **2**, panel A). Therefore, the construct encodes almost a complete 5' UTR of the human acid α-glucosidase gene. This fragment was fused to the promoter sequences of the bovine αS1-casein gene. The αS1-casein initiation site is present 22 bp upstream of the αS1-casein/acid α-glucosidase junction. The construct has the human acid α-glucosidase polyadenylation signal and 3' flanking sequences. Construct c8αgluex2 contains the bovine αS1-casein promoter immediately fused to the translation initiation site in exon 2 of the human acid α-glucosidase gene (see FIG. **2**, panel B). Thus, the αS1-casein transcription initiation site and the α-glucosidase translation initiation site are 22-bp apart in this construct. Therefore no α-glucosidase 5' UTR is preserved. This construct also

contains the human acid α-glucosidase polyadenylation signal and 3' flanking sequences as shown in FIG. **2**, panel B.

Construct c8,8αgluex2-20 differs from construct c8αgluex2 only in the 3' region. A SphI site in exon 20 was used to fuse the bovine αS1-casein 3' sequence to the human acid α-glucosidase construct. The polyadenylation signal is located in this 3' αS1-casein sequence (FIG. **2**, panel C).

Construct c8,8αgluex2-20 differs from construct c8αgluex2 only in the 3' region. A SphI site in exon 20 was used to fuse the bovine αS1-casein 3' sequence to the human acid α-glucosidase construct. The polyadenylation signal is located in this 3' αS1 -casein sequence (FIG. **2**, panel C).

Methods for Construction of Genomic Constructs

Three contiguous BglII fragments containing the human acid α-glucosidase gene were isolated by Hoefsloot et al., supra. These fragments were ligated in the BglII-site of pKUN8ΔC, a pKUN7ΔC derivative with a customized polylinker: HindIII ClaI StuI SstI BglII PvnI NcoI EcoRI SphI XhoI EcoRI* SmaI/SfiI NotI EcoRI* (*=destroyed site). This ligation resulted in two orientations of the fragments generating plasmids p7.3αgluBES, p7.3αgluBSE, p8.5αgluBSE, p8.5αgluBES, p10αgluBSE and p10αgluBES.

Because unique NotI-sites at the ends of the expression cassette are used subsequently for the isolation of the fragments used for microinjection, the intragenic NotI site in intron 17 of human acid α-glucosidase had to be destroyed. Therefore, p10αgluBES was digested with ClaI and XhoI; the fragment containing the 3' α-glucosidase end was isolated. This fragment was inserted in the ClaI and XhoI sites of pKUN10ΔC, resulting in p10αgluΔNV. Previously pKUN10ΔC (i.e., a derivative of pKUN8ΔC) was obtained by digesting pKUN8ΔC with NotI, filling in the sticky ends with Klenow and subsequently, annealing the plasmid by blunt-ended ligation. Finally, p10αgluΔNV was digested with NotI. These sticky ends were also filled with Klenow and the fragment was ligated, resulting in plasmid p10αgluΔNotI.

Construction of c8αgluex1

Since the SstI site in first exon of the α-glucosidase gene was chosen for the fusion to the bovine αS1-casein sequence, p8.5αgluBSE was digested with BglII followed by a partial digestion with SstI. The fragment containing exon 1-3 was isolated and ligated into the BglII and SstI sites of pKUN8ΔC. The resulting plasmid was named: p5'αgluex1. (see FIG. **3**, panel A).

The next step (FIG. **3**, panel B) was the ligation of the 3' part to p5'αgluex1. First, p10αgluΔN was digested with BglII and BamHI. This fragment containing exon 16-20 was isolated. Second, p5'αgluex1 was digested with BglII and to prevent self-ligation, and treated with phosphorylase (BAP) to dephosphorylate the sticky BglII ends. Since BamHI sticky ends are compatible with the BglII sticky ends, these ends ligate to each other. The resulting plasmid, i.e., p5'3'αgluex1, was selected. This plasmid has a unique BglII site available for the final construction step (see FIG. **3**, panels B and C).

The middle part of the α-glucosidase gene was inserted into the latter construct. For this step, p7.3αgluBSE was digested with BglII, the 8.5-kb fragment was isolated and ligated to the BglII digested and dephosphorylated p5'3'αgluex1 plasmid. The resulting plasmid is pαgluex1 (FIG. **3**, panel C).

The bovine αS1-casein promoter sequences were incorporated in the next step via a ligation involving three fragments. The pWE15 cosmid vector was digested with NotI and dephosphorylated. The bovine αS1-casein promoter was isolated as an 8 Rb NotI-ClaI fragment (see de Boer et al., 1991, supra). The human acid α-glucosidase fragment was isolated from pαgluex1 using the same enzymes. These three fragments were ligated and packaged using the Stratagene GigapackII kit in 1046 *E. coli* host cells. The resulting cosmid c8αgluex1 was propagated in *E. coli* strain DH5α. The vector was linearized with NotI before microinjection.

Construction of c8αgluex2 and c8.8αgluex2-20

The construction of the other two expression plasmids (FIG. **2**, panels B and C) followed a similar strategy to that of c8αgluex1. The plasmid p5'αgluStuI was derived from p8,5αgluBSE by digestion of the plasmid with StuI, followed by self-ligation of the isolated fragment containing exon 2-3 plus the vector sequences. Plasmid p5'αgluStuI was digested with PglII followed by a partial digestion of the linear fragment with NcoI resulting in several fragments. The 2.4 kb fragment, containing exon 2 and 3, was isolated and ligated into the NcoI and BglII sites of vector pKUN12ΔC, resulting in p5'αgluex2. Note that pKUN12ΔC is a derivative of pKUN8ΔC containing the polylinker: ClaI NcoI BglII Hindi EcoRI SphI XhoI SmaI/SfiI NotI.

The plasmid p10αgluΔNotI was digested with BglII and HindIII. The fragment containing exons 16-20 was isolated and ligated in p5'αgluex2 digested with BglII and HindIII. The resulting plasmid was p5'3'αgluex2. The middle fragment in p5'3'αgluex2 was inserted as for pαgluex1. For this, p7.3αglu was digested with BglII. The fragment was isolated and ligated to the BglII-digested and dephosphorylated p5'3'αgluex2. The resulting plasmid, pαgluex2, was used in construction of c8αgluex-20 and c8,8αgluex2-20 (FIG. **2**, panels B and C).

For the construction of third expression plasmid c8,8α gluex2-20 (FIG. **2**, panel C) the 3' flanking region of α-glucosidase was deleted. To achieve this, pαgluex2 was digested with SphI. The fragment containing exon 2-20 was isolated and self-ligated resulting in pαgluex2-20. Subsequently, the fragment containing the 3' flanking region of bovine αS1-casein gene was isolated from p16.8αglu by digestion with SphI and NotI. This fragment was inserted into pαgluex2-20 using the SpII site and the NotI site in the polylinker sequence resulting in pαgluex2-20-3αS 1.

The final step in generating c8,8αgluex2-20 was the ligation of three fragments as in the final step in the construction leading to c8αgluex1. Since the ClaI site in pαgluex2-20-3'αS1 and pαgluex2 appeared to be uncleavable due to methylation, the plasmids had to be propagated in the *E. coli* DAM(−) strain ECO343. The pαgluex2-20-3'αS1 isolated from that strain was digested with ClaI and NotI. The fragment containing exons 2-20 plus the 3' αS1-casein flanking region was purified from the vector sequences. This fragment, an 8 kb NotI-ClaI fragment containing the bovine αS1 promoter (see DeBoer (1991) & (1993), supra) and NotI-digested, dephosphorylated pWE15 were ligated and packaged. The resulting cosmid is c8,8αgluex2-20.

Cosmid c8αgluex2 (FIG. **2**, panel B) was constructed via a couple of different steps. First, cosmid c8,8αgluex2-20 was digested with SalI and NotI. The 10.5-kb fragment containing the αS1-promoter and the exons 2-6 part of the acid α-glucosidase gene was isolated. Second, plasmid pαgluex2 was digested with SalI and NotI to obtain the fragment containing the 3' part of the acid α-glucosidase gene. Finally, the cosmid vector pWE15 was digested with

NotI and dephosphorylated. These three fragments were ligated and packaged. The resulting cosmid is c8αgluex2.

Example 2

Transgenesis

The cDNA and genomic constructs were linearized with NotI and injected in the pronucleus of fertilized mouse oocytes which were then implanted in the uterus of pseudopregnant mouse foster mothers. The offspring were analyzed for the insertion of the human α-glucosidase cDNA or genomic DNA gene construct by Southern blotting of DNA isolated from clipped tails. Transgenic mice were selected and bred.

The genomic constructs linearized with NotI were also injected into the pronucleus of fertilized rabbit oocytes, which were implanted in the uterus of pseudopregnant rabbit foster mothers. The offspring were analyzed for the insertion of the alpha-glucosidase DNA by Southern blotting. Transgenic rabbits were selected and bred.

Example 3

Analysis of Acid α-glucosidase in the Milk of Transgenic Mice

Milk from transgenic mice and nontransgenic controls was analyzed by Western Blotting. The probe was mouse antibody specific for human acid α-glucosidase (i.e., does not bind to the mouse enzyme). Transgenes 1672 and 1673 showed expression of human acid α-glucosidase in milk (FIG. **4**). Major and minor bands at 100-110 kD and 76 kD were observed as expected for α-glucosidase. In milk from non-transgenic mice, no bands were observed.

The activity of human acid α-glucosidase was measured with the artificial substrate 4-methylumbelliferyl-α-D-glucopyranoside in the milk of transgenic mouse lines (See Galiaard, Genetic Metabolic Disease, Early Diagnosis and Prenatal Analysis, Elsevier/North Holland, Amsterdam, pp. 809-827 (1980)). Mice containing the cDNA construct (FIG. **1**) varied from 0.2 to 2 μmol/ml per hr. The mouse lines containing the genomic construct (FIG. **2**, panel A) expressed at levels from 10 to 610 μmol/ml per hr. These figures are equivalent to a production of 1.3 to 11.3 mg/l (cDNA construct) and 0.05 to 3.3 g/l (genomic construct) based on an estimated specific activity of the recombinant enzyme of 180 μmol/mg (Van der Ploeg et al., J. Neurol. 235:392-396 (1988)).

The recombinant acid α-glucosidase was isolated from the milk of transgenic mice, by sequential chromatography of milk on ConA-Sepharose™ and Sephadex™ G200. 7 ml milk was diluted to 10 ml with 3 ml Con A buffer consisting of 10 mM sodium phosphate, pH 6.6 and 100 mM NaCl. A 1:1 suspension of Con A sepharose in Con A buffer was then added and the milk was left overnight at 4° C. with gentle shaking. The Con A sepharose beads were then collected by centrifugation and washed 5 times with Con A buffer, 3 times with Con A buffer containing 1 M NaCl instead of 100 mM, once with Con A buffer containing 0.5 M NaCl instead of 100 mM and then eluted batchwise with Con A buffer containing 0.5 M NaCl and 0.1 M methyl-α-D-mannopyranoside. The acid α-glucosidase activity in the eluted samples was measured using the artificial 4-methyl-umbelliferyl-α-D-glycopyranoside substrate (see above). Fractions containing acid α-glucosidase activity were pooled, concentrated and dialyzed against Sephadex buffer consisting of 20 mM Na acetate, pH 4.5 and 25 mM NaCl, and applied to a Sephadex™ 200 column. This column was run with the same buffer, and fractions were assayed for acid α-glucosidase activity and protein content. Fractions rich in acid α-glucosidase activity and practically free of other proteins were pooled and concentrated. The method as described is essentially the same as the one published by Reuser et al., Exp. Cell Res. 155:178-179 (1984). Several modifications of the method are possible regarding the exact composition and pH of the buffer systems and the choice of purification steps in number and in column material.

Acid α-glucosidase purified from the milk was then tested for phosphorylation by administrating the enzyme to cultured fibroblasts from patients with GSD II (deficient in endogenous acid α-glucosidase). In this test mannose 6-phosphate containing proteins are bound by mannose 6-phosphate receptors on the cell surface of fibroblasts and are subsequently internalized. The binding is inhibited by free mannose 6-phosphate (Reuser et al., Exp. Cell Res. 155:178-189 (1984)). In a typical test for the phosphorylation of acid α-glucosidase isolated from milk of transgenic mice, the acid α-glucosidase was added to 104-106 fibroblasts in 500 μl culture medium (Ham F10, supplied with 10% fetal calf serum and 3 mM Pipes) in an amount sufficient to metabolize 1 μmole 4-methyl-umbelliferyl-α-D-glucopyranoside per hour for a time period of 20 hours. The experiment was performed with or without 5 mM mannose 6-phosphate as a competitor, essentially as described by Reuser et al., supra (1984). Under these conditions acid α-glucosidase of the patient fibroblasts was restored to the level measured in fibroblasts from healthy individuals. The restoration of the endogenous acid α-glucosidase activity by acid α-glucosidase isolated from mouse milk was as efficient as restoration by acid α-glucosidase purified from bovine testis, human urine and medium of transfected CHO cells. Restoration by α-glucosidase form milk was inhibited by 5 mM mannose 6-phosphate as observed for α-glucosidase from other sources. (Reuser et al., supra; Van der Ploeg et al., (1988), supra; Van der Ploeg et al., Ped. Res. 24:90-94 (1988).

As a further demonstration of the authenticity of α-glucosidase produced in the milk, the N-terminal amino acid sequence of the recombinant α-glucosidase produced in the milk of mice was shown to be the same as that of α-glucosidase precursor from human urine as published by Hoefsloot et al., EMBO J. 7:1697-1704 (1988) which starts with AHPGRP (SEQ ID NO:1).

Example 4

Animal Trial of Alpha-Glucosidase

Recently, a knock-out mouse model for Pompe's disease has become available (25) This model was generated by targeted disruption of the murine alpha-glucosidase gene. Glycogen-containing lysosomes are detected soon after birth in liver, heart and skeletal muscle. Overt clinical symptoms only become apparent at relatively late age (>9 months), but the heart is typically enlarged and the electrocardiogram is abnormal.

Experiments have been carried out using the knock-out (KO) mouse model in order to study the in vivo effect of AGLU purified from transgenic rabbit milk. The recombinant enzyme in these experiments was purified from milk of the transgenic rabbits essentially as described above for purification from transgenic mice.

1. Short Term Studies in KO Mouse Model

Single or multiple injections with a 6 day interval were administered to KO mice via the tail vein. Two days after the last enzyme administration the animals were killed, and the organs were perfused with phosphate buffered saline (PBS).

main organs involved in uptake, but also the heart and pectoral and femoral muscles take up significant amounts of enzyme. The absence of a significant increase in brain tissue suggests that AGLU does not pass the blood-brain barrier. The results are summarized in Table 2.

TABLE 2

Tissue Uptake of AGLU and Glycogen Content Following Short Term Treatment in KO Mouse Model

	Liver		Spleen		Heart		Pectoral Muscle		Femoral Muscle		Tongue		Brain	
Group	Act	Glc	Act	Glc	Act	Glc	Act	Glc	act	Glc	Act	Glc	Act	Glc
Experiment A animals treated with single dose of 1.7 mg AGLU (from 2 sources)														
treated KO	674	—	—	—	263	—	—	—	24	—	—	—	0.8	—
mice source 1	410				17				3.1				0.4	
treated KO	454	—	—	—	76	—	—	—	12	—	—	—	0.8	—
mice source 2	604				48				10				0.4	
untreated KO mouse	3.1	—	—	—	0.2	—	—	—	0.2	—	—	—	0.2	—
untreated	58	—	—	—	23	—	—	—	11	—	—	—	57	—
normal mouse	37				17				8.2				57	
Experiment B animals treated with 4 doses of AGLU (1.0, 2.0, 1.0 and 1.4 mg) 6 days apart														
treated KO	1132	70	—	—	24	1259	125	87	—	—	89	—	0.4	163
mice (13 weeks old)	944	13			10	1082	46	116			35		0.2	163
treated KO mice (34 weeks old)	3375	23	—	—	60	1971	49	90	—	—	207	—	0.7	374
untreated KO	2.0	406	—	—	0.2	3233	1.0	86	—	—	1.0	—	0.2	487
mice (13 and 34 weeks old)	2.0	147			0.3	1748	1.0	87			1.0		0.2	168
untreated normal mice (34 weeks old)	35	6	—	—	8.2	0	6.0	1.0	—	—	14	—	18	0
Experiment C animals treated with single dose of 0.7 mg														
treated KO	582	—	462	—	46	—	—	—	5.1	—	—	—	0.4	—
mice	558		313		50				3.6				0.4	
untreated KO	1.1	—	0.8	—	0.3	—	—	—	0.2	—	—	—	0.2	—
mice	1.6		0.7		0.3				0.3				0.2	

Figures in the table refer to individual animals
Act: AGLU activity (nmoles 4 MU per mg protein per hour)
Glc: Glycogen content (μg/mg protein)
n.d. not detected
— data unavailable

Tissue homogenates were made for GLU enzyme activity assays and tissue glycogen content, and ultrathin sections of various organs were made to visualize accumulation (via electron microscopy) lysosomal glycogen content. Also the localization of internalized AGLU was determined using rabbit polyclonal antibodies against human placenta mature α-glucosidase.

The results showed that single doses of 0.7 and 1.7 mg AGLU (experiments C and A respectively) was taken up efficiently in vivo in various organs of groups of two knock-out mice when injected intravenously. Experiment A also showed that there were no differences in the uptake and distribution of AGLU purified from two independent rabbit milk sources.

Increases in AGLU activity were seen in the organs such as the liver, spleen, heart, and skeletal muscle, but not in the brain. Two days after a single injection of 1.7 mg AGLU to two KO animals, levels close to, or much higher than, the endogenous alpha-glucosidase activity levels observed in organs of two PBS-injected normal control mice or two heterozygous KO mice were obtained (experiment A). Of the organs tested, the liver and spleen are, quantitatively, the

When two KO mice were injected 4 times every 6 days (experiment B), a marked decrease of total cellular glycogen was observed in both heart and liver. No effects were observed in skeletal muscle tissues with regard to total glycogen. However, in general the uptake of AGLU in these tissues was lower than in the other tissues tested.

Transmission electron microscopy of the 4 times injected KO mice indicated a marked decrease in lysosomal glycogen in both liver cells and heart muscle cells. The effects observed in heart tissue are localized since in some areas of the heart no decrease in lysosomal glycogen was observed after these short term administrations.

Western blot analysis using rabbit polyclonal antibodies against human placenta mature alpha-glucosidase indicated complete processing of the injected AGLU towards the mature enzyme in all organs tested strongly suggesting uptake in target tissues, and lysosomal localization and processing. No toxic effects were observed in any of the three experiments.

Immunohistochemical staining of AGLU was evident in lysosomes of hepatocytes using a polyclonal rabbit antibody against human alpha-glucosidase. The presence of AGLU in

heart and skeletal tissues is more difficult to visualize with this technique, apparently due to the lower uptake.

2. Long-Term Experiments with the KO Mouse Model

In longer term experiments, enzyme was injected in the tail vein of groups of two or three KO mice, once a week for periods of up to 25 weeks. The initial dose was 2 mg (68 mg/kg) followed by 0.5 mg (17 mg/kg)/mouse for 12 weeks. In two groups of mice, this was followed by either 4 or 11 additional weeks of treatment of 2 mg/mouse. Injections started when the mice were 6-7 months of age. At this age, clear histopathology has developed in the KO model. Two days after the last enzyme administration the animals were killed, and the organs were perfused with phosphate buffered saline (PBS). Tissue homogenates were made for AGLU enzyme activity assays and tissue glycogen content, and sections of various organs were made to visualize (via light microscopy) lysosomal glycogen accumulation.

The results showed that mice treated 13 weeks with 0.5 mg/mouse (Group A, 3 animals/Group) had an increase of activity in the liver and spleen and decreased levels of glycogen in liver and perhaps in heart. One animal showed increased activity in muscles, although there was no significant decrease of glycogen in muscle.

Mice that were treated 14 weeks with 0.5 mg/mouse followed by 4 weeks with 2 mg/mouse (Group B, 3 animals/Group) showed similar results to those treated for 13 weeks only, except that an increased activity was measured in the heart and skeletal muscles and decreases of glycogen levels were also seen in the spleen.

Mice that were treated 14 weeks with 0.5 mg/mouse followed by 11 weeks with 2 mg/mouse (Group C 2 animals/Group) showed similar results to the other two groups except that treated mice showed definite decreases in glycogen levels in liver, spleen, heart and skeletal muscle. No activity could be detected, even at the highest dose, in the brain.

Results of treated and untreated animals in each Group (Group means) are summarized in Table 3.

TABLE 3

Tissue Uptake of AGLU and Glycogen Content Following Long Term Treatment in KO Mouse Model.

	Liver		Spleen		Heart		Pectoral Muscle		Quadriceps Muscle		Gastrocnemius Muscle		Brain	
Group	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc
Group A animals treated with 0.5 mg/mouse/week for 13 weeks														
treated	713	2	463	n.d	3	86	9	81	6	40	14	66	—	—
untreated	2	24	1	n.d.	1	111	1	66	1	50	1	61	—	—
Group B animals treated with 0.5 mg/mouse/week for 14 weeks, followed by 2 mg/mouse./week for 4 weeks														
treated	2705	1	1628	0	59	288	49	120	30	128	44	132	—	—
untreated	3	11	31	6	1	472	1	113	1	162	1	142	—	—
Group C animals treated with 0.5 mg/mouse/week for 14 weeks, followed by 2 mg/mouse./week for 11 weeks														
treated	1762	1	1073	2	66	211	99	113	37	18	109	32	1	32
untreated	2	45	1	21	1	729	1	291	0	104	0	224	0	44

Figures in the table refer to the mean of 3 animals (Groups A and B) or the mean of 2 animals (Group C)
Act AGLU activity (nmoles 4 MU per mg protein per hour)
Glc: Glycogen content (μg/mg protein)
n.d. not detected
— data unavailable

In addition, a very convincing improvement in the histopathological condition was observed in Group C mice (treated for the first 14 weeks at 0.5 mg/mouse, followed by 11 weeks at 2 mg/mouse). Clear reversal of pathology was demonstrated in various tissues, such as heart and pectoralis muscle.

It has been reported that Pompe's disease does not occur when the residual lysosomal α-glucosidase activity is >20% of average control value (14). The data obtained with the KO mouse model indicates that these levels are very well achievable using recombinant precursor enzyme.

Example 5

Human Clinical Trial

A single phase I study (AGLU 1101-01) has been conducted in 15 healthy male volunteers. Doses of AGLU ranged from 25 to 800 mg, administered by intravenous infusion to healthy male adult volunteers. Subjects with a history of allergies and hypersensitivities were excluded from the study. The subjects were randomized into dose groups of 5, and each dose Group received AGLU (4 subjects) or placebo (1 subject) at each dose level. All subjects received two doses of study drug, which were administered two weeks apart. The dosing regimen was as follows:

A
25 mg: Group 1, treatment period 1
B
50 mg: Group 1, treatment period 2
C
100 mg: Group 2, treatment period 1
D
200 mg: Group 3, treatment period 1
E
400 mg: Group 2, treatment period 2
F
800 mg: Group 3, treatment period 2
P
placebo (1 subject per Group and treatment period)

Subjects were administered AGLU or placebo as an infusion on Day 1 of each treatment period. The infusions

were administered over a 30 minute period and subjects were kept in a semi-recumbent position for at least 2 hours after cessation of infusion.

Adverse events were recorded just before the start of the infusion, at 30 minutes (end of infusion) and at 3, 12, 24, 36 and 48 hours thereafter as well as on Days 5 and 8 (first period) and days 5, 8 and 15 (second period). Vital signs, ECG and physical examinations were also monitored regularly throughout the treatment period.

Blood samples were taken for a standard range of clinical laboratory tests and pharmacokinetics analysis. The subject's urine was collected and a standard range of laboratory analyses (including determination of AGLU) were performed.

(a) Laboratory Safety and Adverse Events

There were no clinically significant changes in laboratory parameters, clinical signs and ECG measurements in any subjects at any dose Group. The results of adverse event monitoring in all subjects at all doses are summarized in Table 4.

TABLE 4

Adverse Event Reports

Dose (mg)	Adverse Events
25	The reported events were muscle weakness, abnormal vision and fatigue. All events were mild and were deemed unrelated to the test article by the investigator.
50	The reported events were headache, rhinitis, nose bleed and paresthesia. All events were mild and were deemed unrelated or remotely related to the test article by the investigator, except the paresthesia which was classed as moderate and was deemed possibly related to the test article.
100	The reported events were rhinitis, headache, fatigue, hematoma and injection site reaction. All events were classed as mild. The cases of hematoma, injection site reaction and intermittent headache were deemed possibly or probably related to the test article by the investigator. The other events were deemed to be unrelated.
200	The reported events were nausea, headache, dizziness, fatigue, rhinitis, photophobia, vision abnormalities and euphoria. All events were classed as mild or moderate in intensity. Seven events (including cases of dizziness, nausea and abnormal vision) were deemed to be possibly or probably related related to the test article.
400	The reported events were fatigue and paresthesia. The report of fatigue was considered unrelated to the test article, and the paresthesia was deemed possibly related.
800	The reported events were nausea, drowsiness, dizziness, increased sweating, asthenia, shivering and intermittent headache. All events were classed as mild or moderate in intensity. Eight events (including cases of drowsiness, nausea and asthenia) were deemed to be possibly related to the test article.

A trial of the safety and efficacy of recombinant acid α-glucosidase as enzyme replacement therapy on infantile and juvenile patients with glycogen storage disease Type II is conducted. Four infantile patients and three juvenile patients are recruited. Infantiles are administered a starting dose of 15-20 mg/kg titrated to 40 mg/kg and juveniles are administered 10 mg/kg. Patients are treated for 24 weeks.

Patients are evaluated by the following parameters.

Standard adverse event reporting including suspected adverse events

Laboratory parameters including hematology, clinical chemistry and antibody detection.

α-glucosidase activity in muscle

Muscle histopathology

12-lead ECG

Clinical condition including neurological examination

Non-parametric PK parameters

Life saving interventions

Infantile patients are evaluated for the following additional parameters.

Left posterior ventricular wall thickness and left ventricular mass index

Neuromotor development

Survival

Glycogen content in muscle

Juvenile patients are evaluated for the following additional parameters.

Pulmonary function

Muscle strength/timed tests and muscle function

PEDI/Rotterdam 9-item scale

The same patients are then subject to additional dosages of alpha glucosidase with infantiles receiving 15, 20, 30 or 40 mg/kg and juveniles: 10 mg/kg for an additional period of 24 weeks and evaluated by the parameters indicated above.

A further phase II clinical trial is performed on eight patients aged<6 months of age within 2 months after diagnosis at a dosage of 40 mg/kg. Patients are treated for 24 weeks and evaluated by the following criteria:

Safety parameters

Laboratory safety data

Adverse event recording

Primary efficacy parameter: survival without life-saving interventions (i.e. mechanical ventilation>24 hr) 6 months past diagnosis in combination with normal or mildly delayed motor function (BSID II).

Secondary efficacy: Changes in neuromotor development, changes in left posterior ventricular wall thickness and left ventricular mass index; Changes in skeletal muscle acid α-glucosidase activity and glycogen content.

Efficacy can be show by a 50% survival at 6 months post-diagnosis without life saving interventions in the α-glucosidase group compared to 10% survival in the historical control group in combination with a BSID II classified as normal or mildly delayed.

A further clinical trial is performed on juvenile patients. The patients are aged>1 year and <35 years of age with juvenile onset of GSD type IIb The patients are administered 10 mg/kg or 20 mg/kg for a period of twenty-four weeks treatment. Treatment is monitored by the following parameters.

Safety parameters	Laboratory safety data Adverse event recording
Primary efficacy	Pulmonary function parameters (e.g. FVC, time on ventilator) Muscle strength
Secondary efficacy	Life-saving interventions parameters: Quality of life Skeletal muscle acid α-glucosidase activity
Quantitative objective	20% relative improvement in primary efficacy parameters over baseline

All quantitative measurements relating to efficacy are preferably statistically significant relative to contemporaneous or historical controls, preferably at $p<0.05$.

Example 6

Pharmaceutical Formulations

Alpha-glucosidase is formulated as follows: 5 mg/ml □-Glu, 15 mM sodium phosphate, pH 6.5, 2% (w/w) mannitol, and 0.5% (w/w) sucrose. The above formulation is filled to a final volume of 10.5 ml into a 20 cc tubing vial and lyophilized. For testing, release and clinical use, each vial is reconstituted with 10.3 ml* of sterile saline (0.9%) for injection (USP or equivalent.) to yield 10.5 ml of a 5 mg/ml □-Glu solution that may be directly administered or subsequently diluted with sterile saline to a patient specific target dose concentration. The 10.5 ml fill (52.5 mg alpha glucosidase total in vial) includes the USP recommended overage, that allows extraction and delivery (or transfer) of 10 mls (50 mg). The mannitol serves as a suitable bulling agent shortening the lyophilization cycle (relative to sucrose alone). The sucrose serves as a cryo/lyoprotectant resulting in no significant increase in aggregation following reconstitution. Reconstitution of the mannitol (only) formulations had repeatedly resulted in a slight increase in aggregation. Following lyophilization, the cake quality was acceptable and subsequent reconstitution times were significantly reduced Saline is preferred to HSA/dextrose for infusion solution. When saline is used in combination with lyophilization in 2% mannitol/0.5% sucrose the solution has acceptable tonicity for intravenous administration. The lyophilized vials containing the 2% mannitol/0.5% sucrose formulation were reconstituted with 0.9% sterile saline (for injection) to yield 5 mg/ml □-Glu.

Example 7

Infusion Schedule

The solution is administered via the indwelling intravenous cannula. Patients are monitored closely during the infusion period and appropriate clinical intervention are taken in the event of an adverse event or suspected adverse event. A window of 48 hours is allowed for each infusion. An infusion schedule in which the rate of infusion increases with time reduces or eliminates adverse events.

Infusions for infantiles can be administered according to the following schedule:

5 cc/hr for 60 minutes
10 cc/hr for 60 minutes
≧40 cc/hr for 30 minutes
≧80 cc/hr for the remainder of the infusion

Infusions for juveniles can be administered according to the following schedule:

0.5 cc/kg/hr for 60 minutes
1 cc/kg/hr for 60 minutes
5 cc/kg/hr for 30 minutes
7.5 cc/kg hr for the remainder of the infusion

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

SEQUENCE LISTING

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What is claimed is:

1. A method of treating a human patient with Pompe's disease, comprising intravenously administering biweekly to the patient a therapeutically effective amount of human acid alpha glucosidase, whereby the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,351,410 B2
APPLICATION NO. : 10/611598
DATED : April 1, 2008
INVENTOR(S) : Johannes B.M.M. van Bree, Edna H.G. Venneker and David P. Meeker

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page;

Field (60), page 1 does not list the correct chain of title. Please add the following applications at the end of field (60):

-- Related to U.S. Application No. 08/700,760 filed July 29, 1996, now U.S. Patent No. 6,118,045, which claims the benefit of U.S. Provisional Application No. 60/001,796, filed August 2, 1995 --

Signed and Sealed this

Second Day of September, 2008

JON W. DUDAS
Director of the United States Patent and Trademark Office

US007655226B2

(12) **United States Patent**
Van Bree et al.

(10) **Patent No.: US 7,655,226 B2**
(45) **Date of Patent: *Feb. 2, 2010**

(54) **TREATMENT OF POMPE'S DISEASE**

(75) Inventors: **Johannes B. M. M. Van Bree**, Nieuw-Vennep (NL); **Edna H. G. Venneker**, Saturnushof 15 (NL); **David P. Meeker**, Concord, MA (US)

(73) Assignee: **Genzyme Therapeutic Products Limited Partnership**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 10 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/012,003**

(22) Filed: **Jan. 30, 2008**

(65) **Prior Publication Data**

US 2008/0206223 A1 Aug. 28, 2008

Related U.S. Application Data

(63) Continuation of application No. 10/611,598, filed on Jun. 30, 2003, now Pat. No. 7,351,410, which is a continuation of application No. 09/454,711, filed on Dec. 6, 1999, now abandoned.

(60) Provisional application No. 61/111,291, filed on Dec. 7, 1998, provisional application No. 60/001,796, filed on Aug. 2, 1995.

(51) **Int. Cl.**
A61K 38/46 (2006.01)
A61K 38/43 (2006.01)

(52) **U.S. Cl.** **424/94.61**; 424/94.1; 424/94.6; 435/183; 435/200; 435/201

(58) **Field of Classification Search** 435/183, 435/200, 201; 424/94.1, 94.6, 94.61
See application file for complete search history.

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(Continued)

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(57) **ABSTRACT**

The invention provides methods of treating Pompe's disease using human acid alpha glucosidase. A preferred treatment regime comprises administering greater than 10 mg/kg body weight per week to a patient.

6 Claims, 7 Drawing Sheets

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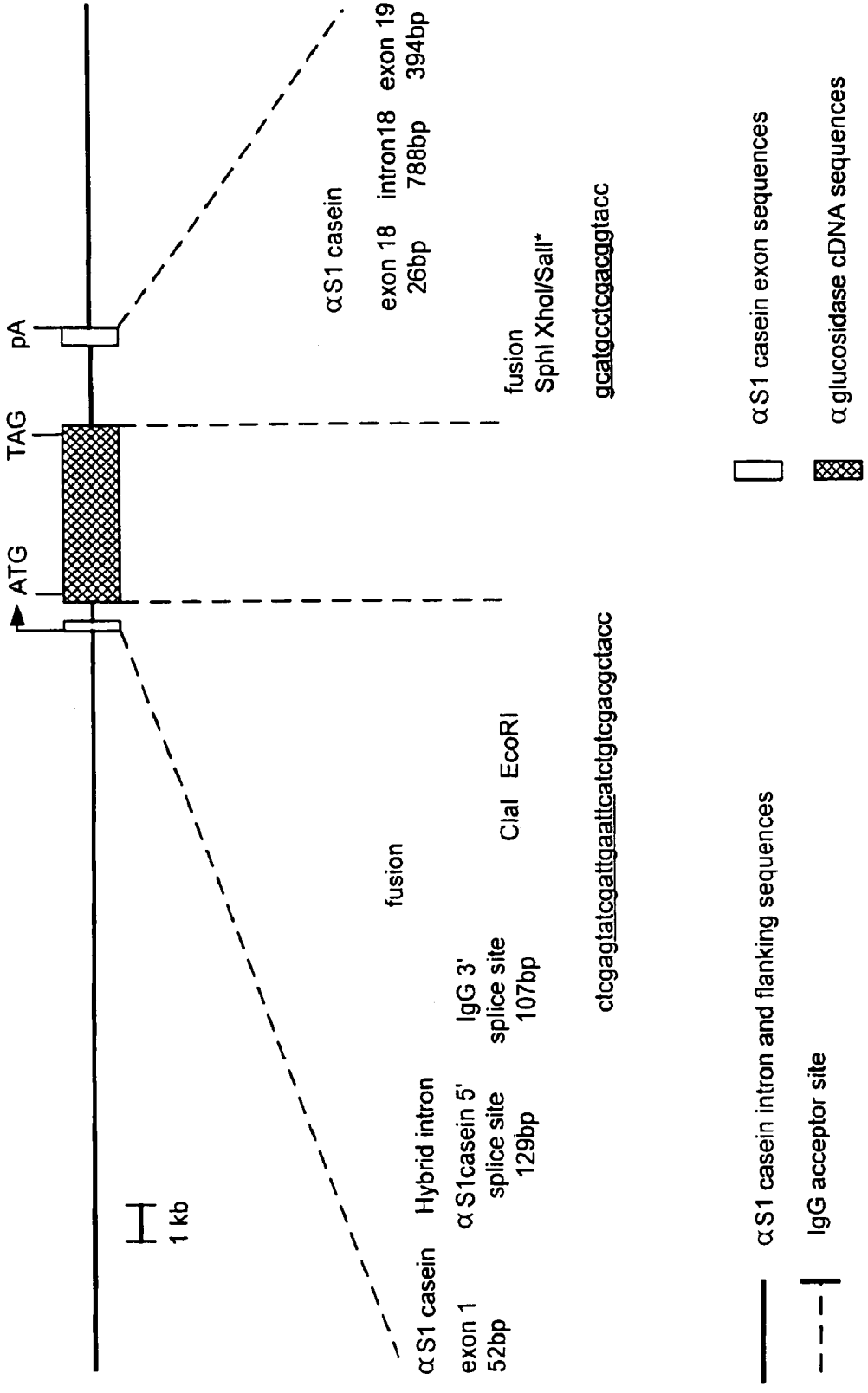
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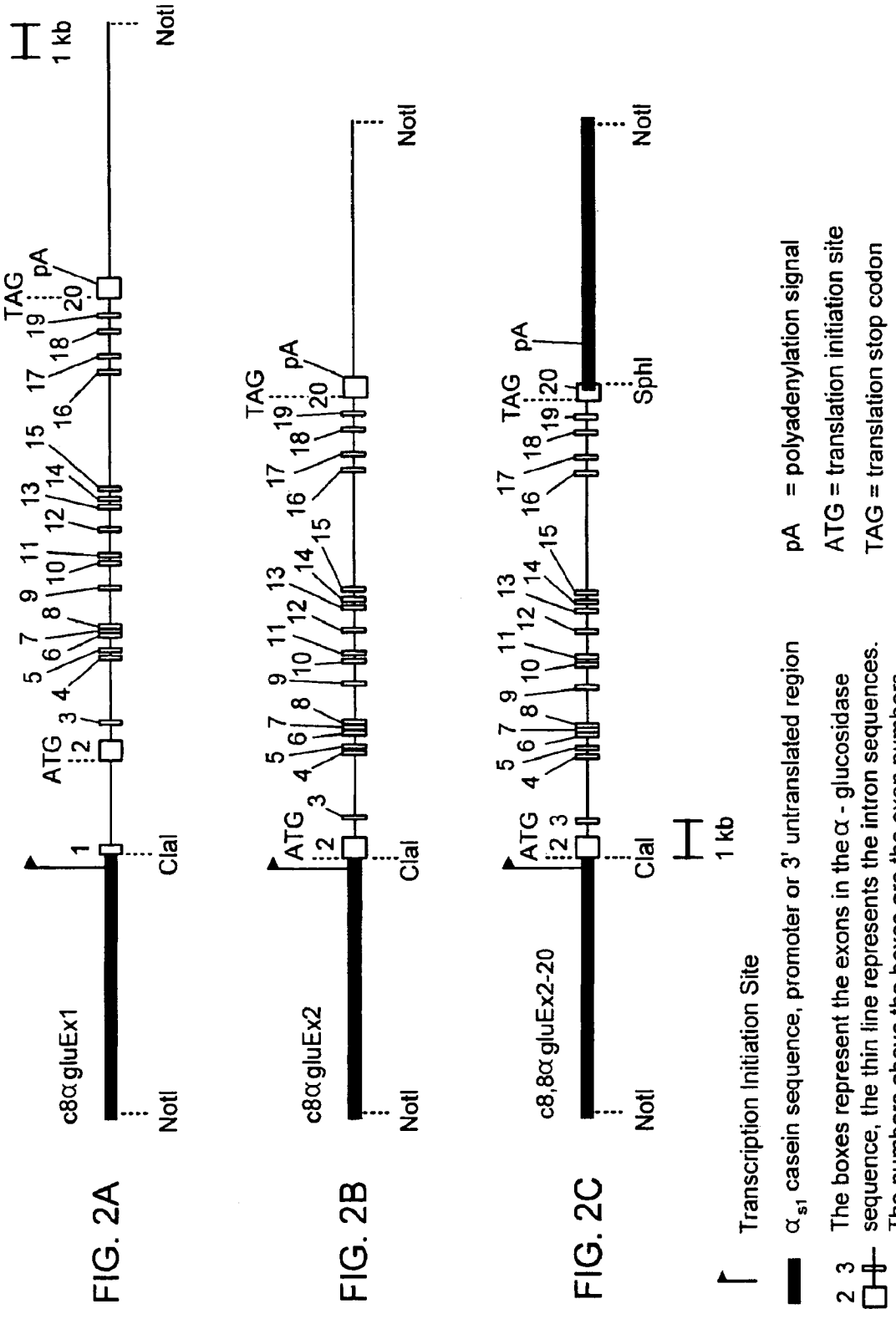
IgG acceptor site

FIG. 1


1 kb
NotI
TAG
pA
ATG
ClaI
c8αgluEx1
FIG. 2A
c8αgluEx2
FIG. 2B
SphI
c8,8αgluEx2-20
FIG. 2C
Transcription Initiation Site
α_{s1} casein sequence, promoter or 3' untranslated region
2 3 The boxes represent the exons in the α - glucosidase sequence, the thin line represents the intron sequences. The numbers above the boxes are the exon numbers.
pA = polyadenylation signal
ATG = translation initiation site
TAG = translation stop codon

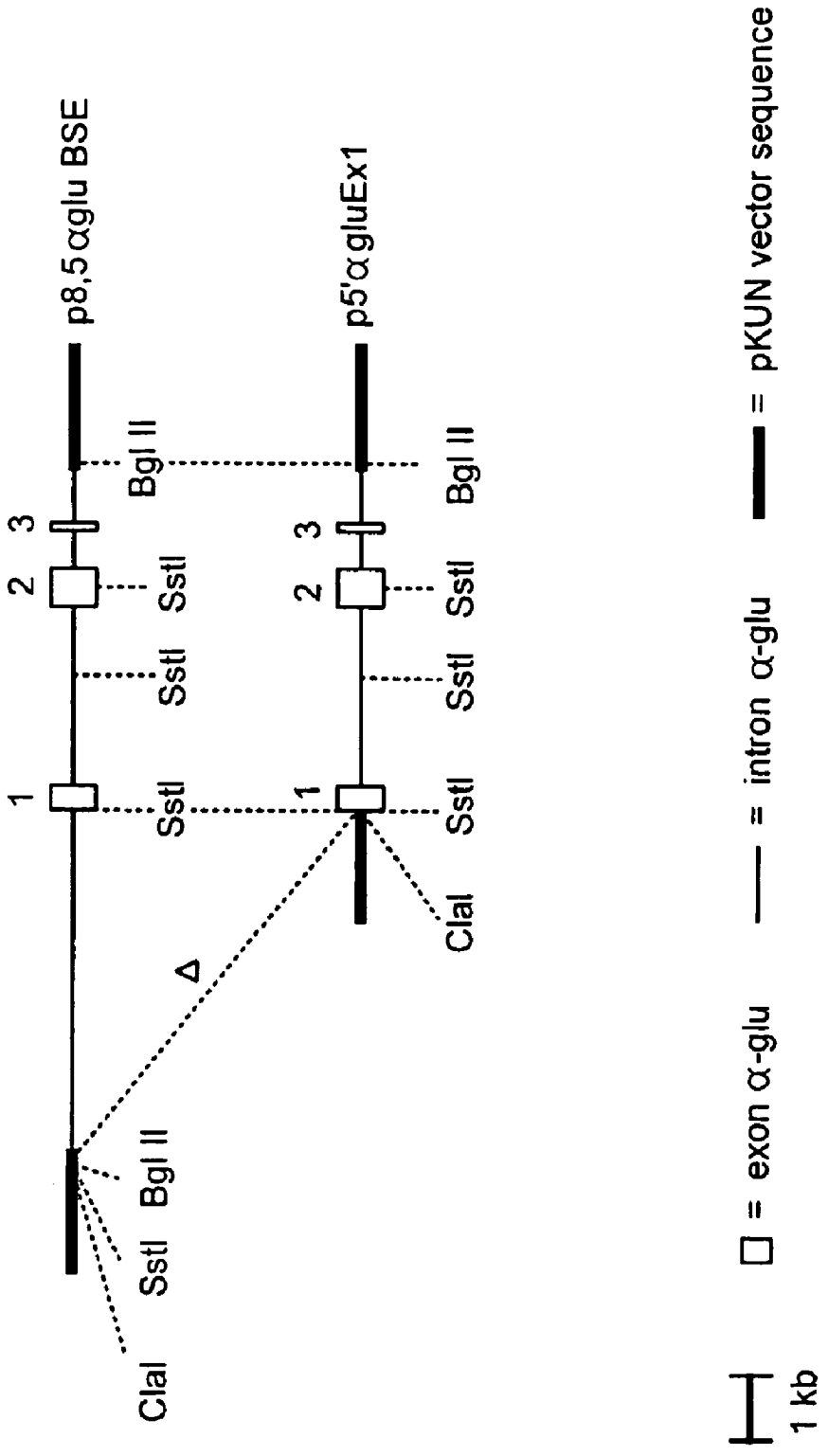

p8,5αglu BSE
p5'αgluEx1
Bgl II
Bgl II
3
2
1
3
2
1
SstI
SstI
SstI
SstI
SstI
SstI
ClaI
ClaI
SstI
Bgl II
Δ
= pKUN vector sequence
= intron α-glu
= exon α-glu
1 kb

FIG. 3A

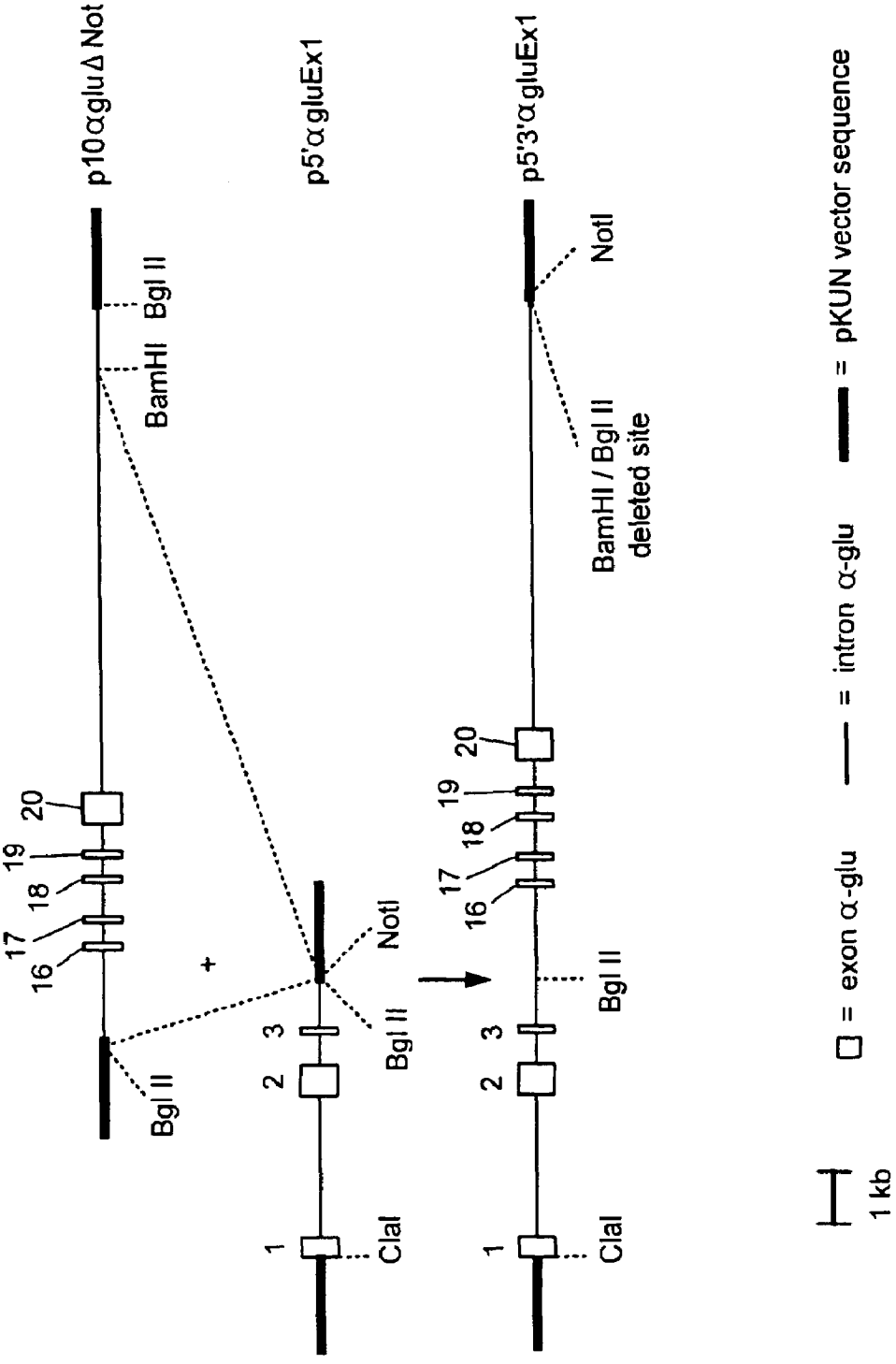

p10αgluΔNot
BglII
BamHI
20
19
18
17
16
BglII
+
p5'αgluEx1
NotI
BglII
3
2
1
ClaI
p5'3'αgluEx1
NotI
BamHI / BglII
deleted site
20
19
18
17
16
BglII
3
2
1
ClaI
□ = exon α-glu
— = intron α-glu
= pKUN vector sequence
1 kb

FIG. 3B

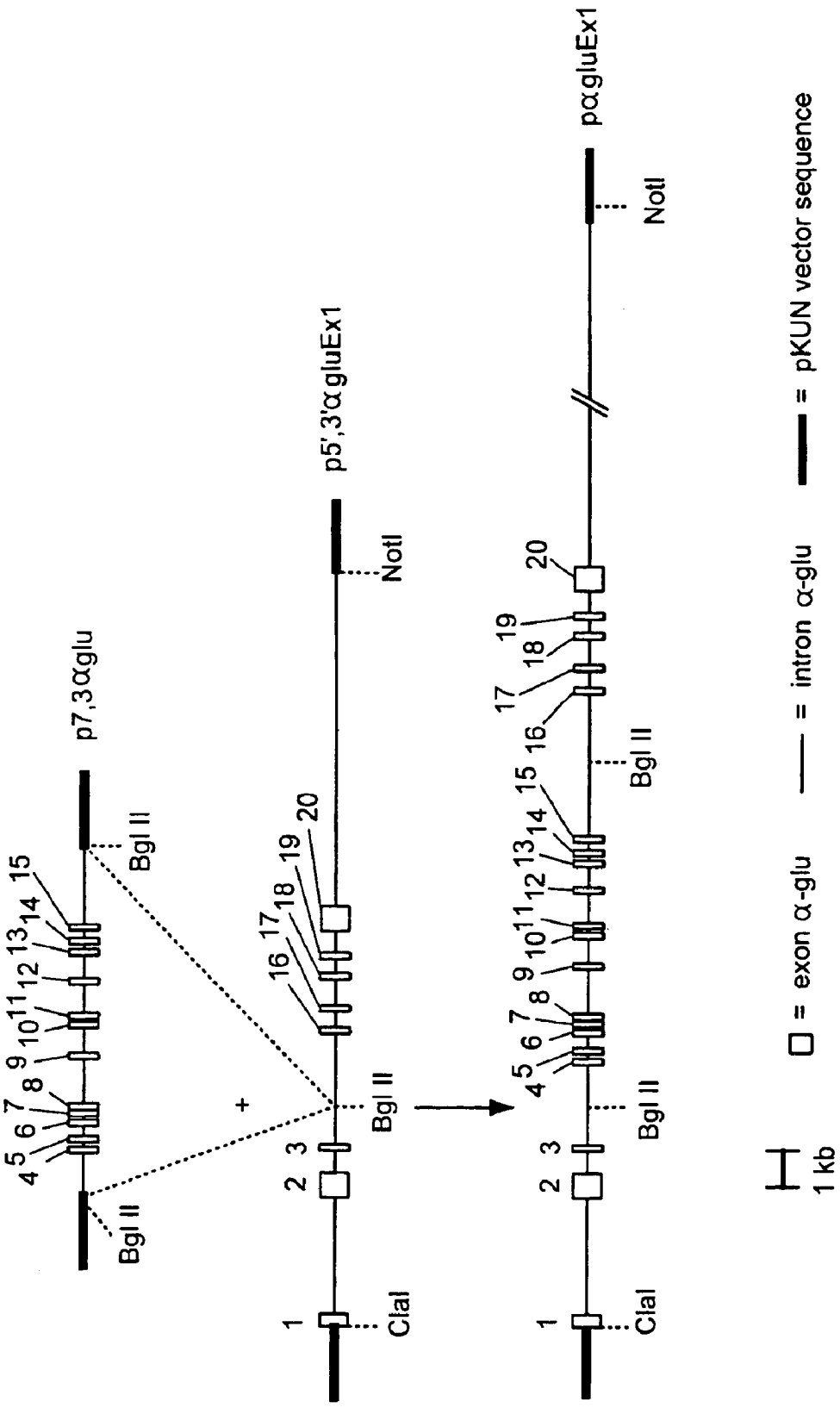

p7,3αglu
Bgl II
Bgl II
p5',3'α gluEx1
NotI
ClaI
pαgluEx1
NotI
Bgl II
Bgl II
ClaI
= exon α-glu
= intron α-glu
= pKUN vector sequence
1 kb

FIG. 3C

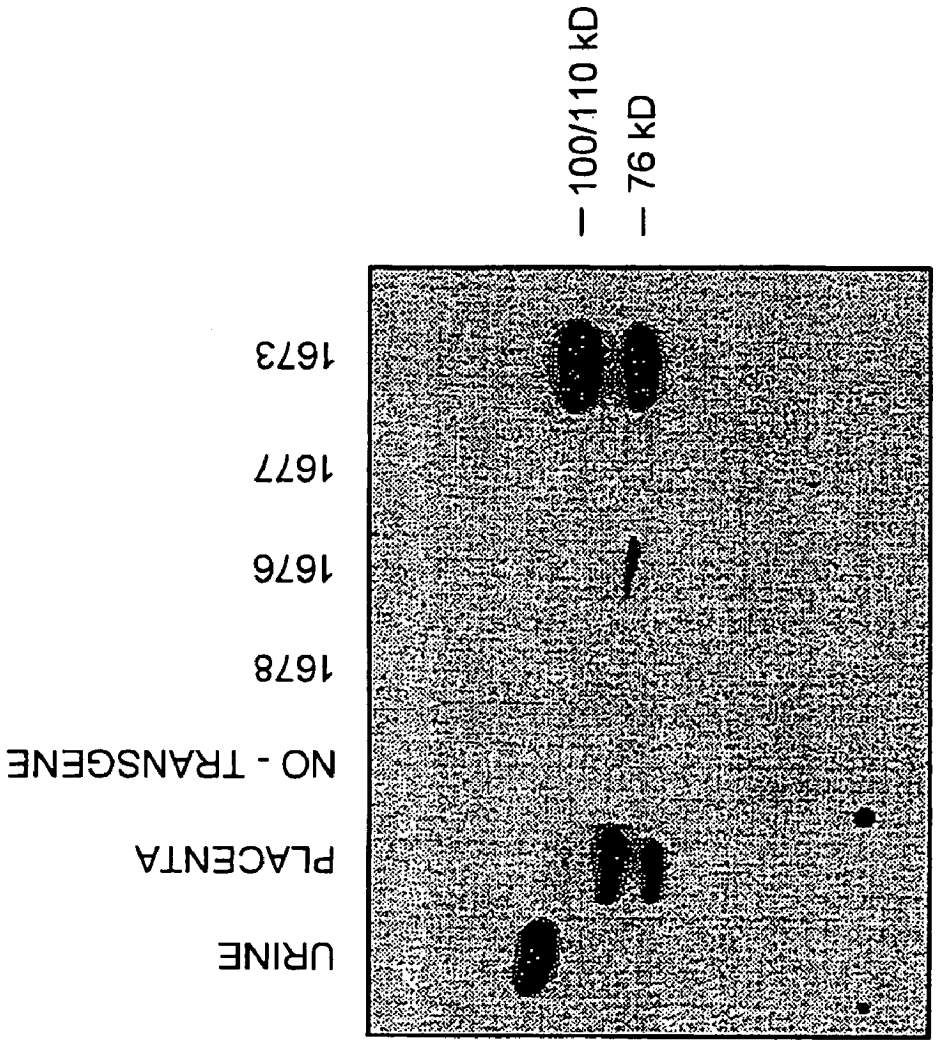

100/110 kD
76 kD
1673
1677
1676
1678
NO - TRANSGENE
PLACENTA
URINE

FIG. 4A

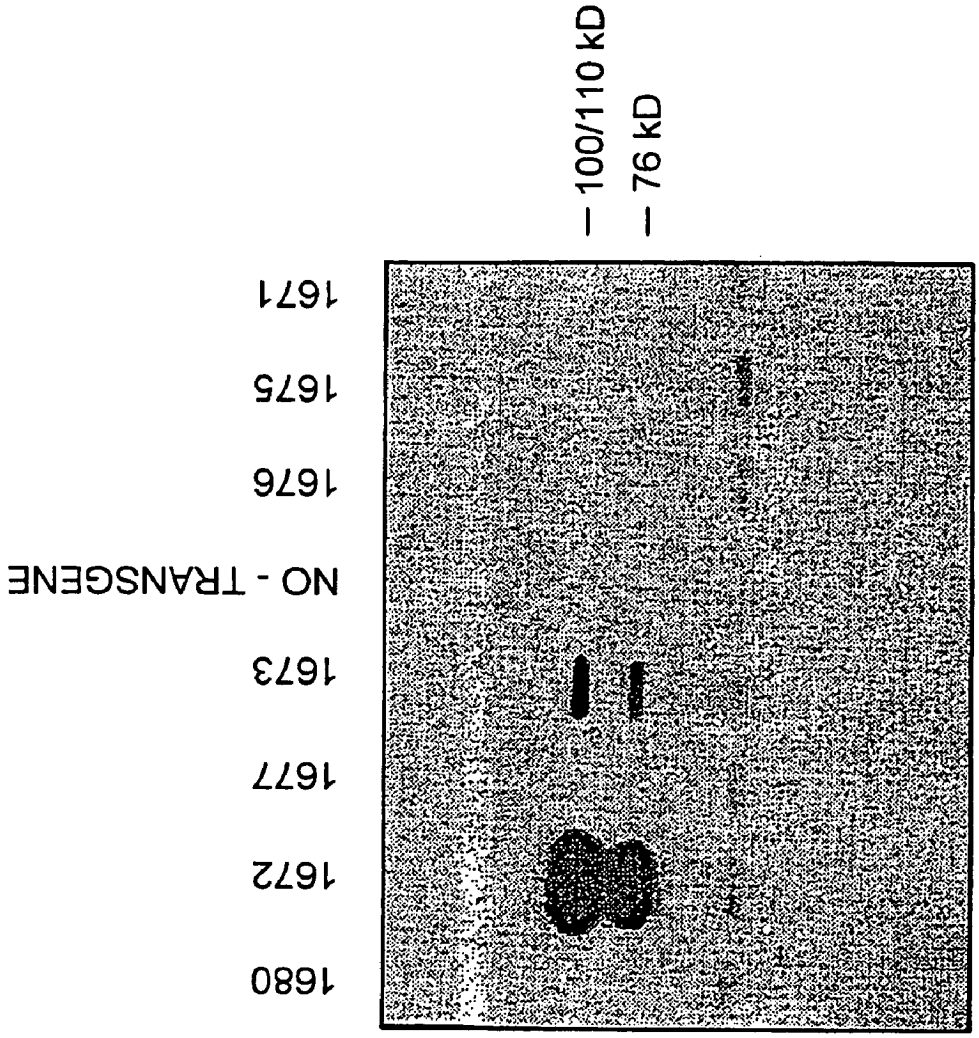

100/110 kD
76 kD
1671
1675
1676
NO - TRANSGENE
1673
1677
1672
1680

FIG. 4B

TREATMENT OF POMPE'S DISEASE

RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 10/611,598, filed Jun. 30, 2003 now U.S. Pat. No. 7,351,410 which is a continuation of U.S. application Ser. No. 09/454,711, filed Dec. 6, 1999, now abandoned which claims the benefit of U.S. Provisional Application No. 60/111,291 filed Dec. 7, 1998, which is related to U.S. application Ser. No. 08/700,760 filed Jul. 29, 1996, now U.S. Pat. No. 6,118,045, which claims the benefit of U.S. Provisional Application No. 60/001,796, filed Aug. 2, 1995. The entire teachings of the above applications are incorporated herein by reference.

TECHNICAL FIELD

The present invention resides in the fields of recombinant genetics, and medicine, and is directed to enzyme-replacement therapy of patients with Pompe's disease.

BACKGROUND OF THE INVENTION

Like other secretory proteins, lysosomal proteins are synthesized in the endoplasmic reticulum and transported to the Golgi apparatus. However, unlike most other secretory proteins, the lysosomal proteins are not destined for secretion into extracellular fluids but into an intracellular organelle. Within the Golgi, lysosomal proteins undergo special processing to equip them to reach their intracellular destination. Almost all lysosomal proteins undergo a variety of posttranslational modifications, including glycosylation and phosphorylation via the 6' position of a terminal mannose group. The phosphorylated mannose residues are recognized by specific receptors on the inner surface of the Trans Golgi Network. The lysosomal proteins bind via these receptors, and are thereby separated from other secretory proteins. Subsequently, small transport vesicles containing the receptor-bound proteins are pinched off from the Trans Golgi Network and are targeted to their intracellular destination. See generally Kornfeld, Biochem. Soc. Trans. 18, 367-374 (1990).

There are over thirty lysosomal diseases, each resulting from a deficiency of a particular lysosomal protein, usually as a result of genetic mutation. See, e.g., Cotran et al., Robbins Pathologic Basis of Disease (4th ed. 1989) (incorporated by reference in its entirety for all purposes). The deficiency in the lysosomal protein usually results in harmful accumulation of a metabolite. For example, in Hurler's, Hunter's, Morquio's, and Sanfilippo's syndromes, there is an accumulation of mucopolysaccharides; in Tay-Sachs, Gaucher, Krabbe, Niemann-Pick, and Fabry syndromes, there is an accumulation of sphingolipids; and in fucosidosis and mannosidosis, there is an accumulation of fucose-containing sphingolipids and glycoprotein fragments, and of mannose-containing oligosaccharides, respectively.

Glycogen storage disease type II (GSD II; Pompe disease; acid maltase deficiency) is caused by deficiency of the lysosomal enzyme acid α-glucosidase (acid maltase). Two clinical forms are distinguished: early onset infantile and late onset, juvenile and adult. Infantile GSD II has its onset shortly after birth and presents with progressive muscular weakness and cardiac failure. This clinical variant is usually fatal within the first two years of life. Symptoms in the late onset in adult and juvenile patients occur later in life, and only skeletal muscles are involved. The patients eventually die due to respiratory insufficiency. Patients may exceptionally survive for more than six decades. There is a good correlation between

the severity of the disease and the residual acid α-glucosidase activity, the activity being 10-20% of normal in late onset and less than 2% in early onset forms of the disease (see Hirschhorn, The Metabolic and Molecular Bases of Inherited Disease (Scriver et al., eds., 7th ed., McGraw-Hill, 1995), pp. 2443-2464).

Since the discovery of lysosomal enzyme deficiencies as the primary cause of lysosomal storage diseases (see, e.g., Hers, Biochem. J. 86, 11-16 (1963)), attempts have been made to treat patients having lysosomal storage diseases by (intravenous) administration of the missing enzyme, i.e., enzyme therapy. These experiments with enzyme replacement therapy for Pompe's disease were not successful. Either non-human α-glucosidase from Aspergillus niger, giving immunological reactions, or a form of the enzyme that is not efficiently taken up by cells (the low uptake form, mature enzyme from human placenta; see below) was used. Moreover, both the duration of treatment, and/or the amount of enzyme administered were insufficient (3-5). Production of lysosomal enzymes from natural sources such as human urine and bovine testis is in theory possible, but gives low yields, and the enzyme purified is not necessarily in a form that can be taken up by tissues of a recipient patient.

Notwithstanding the above uncertainties and difficulties, the invention provides methods of treating patients for Pompe's disease using human acid alpha glucosidase.

SUMMARY OF THE INVENTION

In one aspect, the invention provides methods of treating a patient with Pompe's disease. Such methods entail administering to the patient a therapeutically effective amount of human acid alpha glucosidase. The dosage is preferably at least 10 mg/kg body weight per week. In some methods, the dosage is at least 60 mg/kg body weight per week or at least 120 mg/kg body weight per week. In some methods, such dosages are administered on a single occasion per week and in other methods on three occasions per week. In some methods, the treatment is continued for ate least 24 weeks. Administration is preferably intravenous. The human acid alpha glucosidase is preferably obtained in the milk of a nonhuman transgenic mammal, and is preferably predominantly in a 110 kD form.

The methods can be used for treating patients with infantile, juvenile or adult Pompe's disease. In some methods of treating infantile Pompe's disease efficacy is indicated by a patient surviving to be at least one year old.

In some methods, levels of human acid alpha glucosidase are monitored in the patient. Optionally, a second dosage of human acid alpha glucosidase can be administered if the level of alpha-glucosidase falls below a threshold value in the patient.

In some methods, the human alpha glucosidase is administered intravenously and the rate of administration increases during the period of administration. In some methods, the rate of administration increases by at least a factor of ten during the period of administration. In some methods, the rate of administration increases by at least a factor of ten within a period of five hours. In some methods, the patient is administered a series of at least four dosages, each dosage at a higher strength than the previous dosage. In some methods, the dosages are a first dosage of 0.03-3 mg/kg/hr, a second dosage of 0.3-12 mg/kg/hr, a third dosage of 1-30 mg/kg/hr and a fourth dosage of 2-60 mg/kg/hr. In some methods, the dosages are a first dosage of 0.1-1 mg/kg/hr, a second dosage of 14 mg/kg/hr, a third dosage of 3-10 mg/kg/hr and a fourth dosage of 6-20 mg/kg/hr. In some methods, the dosages are a

first dosage of 0.254 mg/kg/hr, a second dosage of 0.9-1.4 mg/kg/hr, a third dosage of 3.6-5.7 mg/kg/hr and a fourth dosage of 7.211.3 mg/kg/hr. In some methods, the dosages are a first dosage of 0.3 mg/kg/hr, a second dosage of 1 mg/kg/hr, a third dosage of 4 mg/kg/hr and a fourth dosage of 12 mg/kg/hr. In some methods, the first, second, third and fourth dosages are each administered for periods of 15 min to 8 hours.

In some methods, the first, second, third and fourth dosages are administered for periods of 1 hr, 1 hr, 0.5 hr and 3 hr respectively.

In another aspect, the invention provides a pharmaceutical composition comprising human acid alpha glucosidase, human serum albumin, and a sugar in a physiologically acceptable buffer in sterile form. Some such compositions comprise human acid alpha glucosidase, human serum albumin, and glucose in sodium phosphate buffer. Some compositions comprise alpha glucosidase, mannitol and sucrose in an aqueous solution. In some compositions, the sugar comprises mannitol and sucrose and the concentration of mannitol is 1-3% w/w of the aqueous solution and the concentration of sucrose is 0.1 to 1% w/w of the aqueous solution. In some compositions, the concentration of mannitol is 2% w/w and the concentration of sucrose is 0.5% w/w.

The invention further provides a lyophilized composition produced by lyophilizing a pharmaceutical composition comprising human acid glucosidase, mannitol and sucrose in aqueous solution. Such a composition can be prepared by lyophilizing a first composition comprising human acid alpha-glucosidase, mannitol, sucrose and an aqueous solution to produce a second composition; and reconstituting the lyophilized composition in saline to produce a third composition. In some such compositions, the human acid alpha-glucosidase is at 5 mg/ml in both the first and third composition, the mannitol is at 2 mg/ml in the first composition, the sucrose is at 0.5 mg/ml in the first composition, and the saline used in the reconstituting step is 0.9% w/w.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**: A transgene containing acid α-glucosidase cDNA. The αs1-casein exons are represented by open boxes; α-glucosidase cDNA is represented by a shaded box. The αs1-casein intron and flanking sequences (SEQ ID NOS:2 and 3) are represented by a thick line. A thin line represents the IgG acceptor site. The transcription initiation site is marked (1$^{\rightarrow}$), the translation initiation site (ATG), the stop codon (TAG) and the polyadenylation site (pA).

FIG. **2** (panels A, B, C): Three transgenes containing acid α-glucosidase genomic DNA. Dark shaded areas are αs1 casein sequences, open boxes represent acids α-glucosidase exons, and the thin line between the open boxes represents α-glucosidase introns. Other symbols are the same as in FIG. **1**

FIG. **3** (panels A, B, C): Construction of genomic transgenes. The α-glucosidase exons are represented by open boxes; the α-glucosidase introns and nontranslated sequences are indicated by thin lines. The pKUN vector sequences are represented by thick lines.

FIGS. **4**A and **4**B. Detection of acid α-glucosidase in milk of transgenic mice by Western blotting.

DEFINITIONS

The term "substantial identity" or "substantial homology" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 65 percent sequence identity, preferably at least 80 or 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

The term "substantially pure" or "isolated" means an object species has been identified and separated and/or recovered from a component of its natural environment. Usually, the object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 to 90 percent by weight of all macromolecular species present in the composition. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of derivatives of a single macromolecular species.

A DNA segment is operably linked when placed into a functional relationship with another DNA segment. For example, DNA for a signal sequence is operably linked to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it stimulates the transcription of the sequence. Generally, DNA sequences that are operably linked are contiguous, and in the case of a signal sequence both contiguous and in reading phase. However, enhancers need not be contiguous with the coding sequences whose transcription they control. Linking is accomplished by ligation at convenient restriction sites or at adapters or linkers inserted in lieu thereof.

An exogenous DNA segment is one foreign to the cell, or homologous to a DNA segment of the cell but in an unnatural position in the host cell genome. Exogenous DNA segments are expressed to yield exogenous polypeptides.

In a transgenic mammal, all, or substantially all, of the germline and somatic cells contain a transgene introduced into the mammal or an ancestor of the mammal at an early embryonic stage.

DETAILED DESCRIPTION

The invention provides transgenic nonhuman mammals secreting a lysosomal protein into their milk. Secretion is achieved by incorporation of a transgene encoding a lysosomal protein and regulatory sequences capable of targeting expression of the gene to the mammary gland. The transgene is expressed, and the expression product posttranslationally modified within the mammary gland, and then secreted in milk. The posttranslational modification can include steps of glycosylation and phosphorylation to produce a mannose-6 phosphate containing lysosomal protein.

A. Lysosomal Genes

The invention provides transgenic nonhuman mammals expressing DNA segments containing any of the more than 30 known genes encoding lysosomal enzymes and other types of lysosomal proteins, including α-glucosidase, α-L-iduronidase, iduronate-sulfate sulfatase, hexosaminidase A and B, ganglioside activator protein, arylsulfatase A and B, iduronate sulfatase, heparan N-sulfatase, galacto-ceramidase, α-galactosylceramidase A, sphingomyelinase, α-fucosidase, α-mannosidase, aspartylglycosamine amide hydrolase, acid

lipase, N-a cetyl-a-D-glucosamine-6-sulphate sulfatase, α- and β-galactosidase, β-glucuronidase, β-mannosidase, ceramidase, galacto-cerebrosidase, α-N-acetylgalactosaminidase, and protective protein and others. Transgenic mammals expressing allelic, cognate and induced variants of any of the known lysosomal protein gene sequences are also included. Such variants usually show substantial sequence identity at the amino acid level with known lysosomal protein genes. Such variants usually hybridize to a known gene under stringent conditions or crossreact with antibodies to a polypeptide encoded by one of the known genes.

DNA clones containing the genomic or cDNA sequences of many of the known genes encoding lysosomal proteins are available. (Scott et al., Am. J. Hum. Genet. 47, 802-807 (1990); Wilson et al., PNAS 87, 8531-8535 (1990); Stein et al., J. Biol. Chem. 264, 1252-1259 (1989); Ginns et al., Biochem. Biophys. Res. Comm. 123, 574-580 (1984); Hoefsloot et al., EMBO J. 7, 1697-1704 (1988); Hoefsloot et al., Biochem. J. 272, 473-479 (1990); Meyerowitz and Proia, PNAS 81, 5394-5398 (1984); Scriver et al., supra, part 12, pages 2427-2882 and references cited therein)) Other examples of genomic and cDNA sequences are available from GenBank. To the extent that additional cloned sequences of lysosomal genes are required, they may be obtained from genomic or cDNA libraries (preferably human) using known lysosomal protein DNA sequences or antibodies to known lysosomal proteins as probes.

B. Conformation of Lysosomal Proteins

Recombinant lysosomal proteins are preferably processed to have the same or similar structure as naturally occurring lysosomal proteins. Lysosomal proteins are glycoproteins that are synthesized on ribosomes bound to the endoplasmic reticulum (RER). They enter this organelle co-translationally guided by an N-terminal signal peptide (Ng et al., Current Opinion in Cell Biology 6, 510-516 (1994)). The N-linked glycosylation process starts in the RER with the en bloc transfer of the high-mannose oligosaccharide precursor Glc3Man9GlcNAc2 from a dolichol carrier. Carbohydrate chain modification starts in the RER and continue in the Golgi apparatus with the removal of the three outermost glucose residues by glycosidases I and II. Phosphorylation is a two-step procedure in which first N-acetyl-gluco-samine-1-phosphate is coupled to select mannose groups by a lysosomal protein specific transferase, and second, the N-acetyl-glucosamine is cleaved by a diesterase (Goldberg et al., Lysosomes: Their Role in Protein Breakdown (Academic Press Inc., London, 1987), pp. 163-191). Cleavage exposes mannose 6-phosphate as a recognition marker and ligand for the mannose 6-phosphate receptor mediating transport of most lysosomal proteins to the lysosomes (Kornfeld, Biochem. Soc. Trans. 18, 367-374 (1992)).

In addition to carbohydrate chain modification, most lysosomal proteins undergo proteolytic processing, in which the first event is removal of the signal peptide. The signal peptide of most lysosomal proteins is cleaved after translocation by signal peptidase after which the proteins become soluble. There is suggestive evidence that the signal peptide of acid α-glucosidase is cleaved after the enzyme has left the RER, but before it has entered the lysosome or the secretory pathway (Wisselaar et al., J. Biol. Chem. 268, 2223-2231 (1993)). The proteolytic processing of acid at-glucosidase is complex and involves a series of steps in addition to cleavage of the signal peptide taking place at various subcellular locations. Polypeptides are cleaved off at both the N and C terminal ends, whereby the specific catalytic activity is increased. The main species recognized are a {fraction ($^{110}/_{100}$)} kD precursor, a 95 kD intermediate and 76 kD and 70 kD mature forms. (Hasilik et al., J. Biol. Chem. 255, 4937-4945 (1980); Oude Elferink et al., Eur. J. Biochem. 139, 489-495 (1984); Reuser et al., J. Biol. Chem. 260, 8336-8341 (1985); Hoefsloot et al., EMBO J. 7, 1697-1704 (1988)). The post translational processing of natural human acid α-glucosidase and of recombinant forms of human acid α-glucosidase as expressed in cultured mammalian cells like COS cells, BHK cells and CHO cells is similar (Hoefsloot et al., (1990) supra; Wisselaar et al., (1993) supra.

Authentic processing to generate lysosomal proteins phosphorylated at the 6' position of the mannose group can be tested by measuring uptake of a substrate by cells bearing a receptor for mannose 6-phosphate. Correctly modified substrates are taken up faster than unmodified substrates, and in a manner whereby uptake of the modified substrate can be competitively inhibited by addition of mannose 6-phosphate.

C. Transgene Design

Transgenes are designed to target expression of a recombinant lysosomal protein to the mammary gland of a transgenic nonhuman mammal harboring the transgene. The basic approach entails operably linking an exogenous DNA segment encoding the protein with a signal sequence, a promoter and an enhancer. The DNA segment can be genomic, minigene (genomic with one or more introns omitted), cDNA, a YAC fragment, a chimera of two different lysosomal protein genes, or a hybrid of any of these. Inclusion of genomic sequences generally leads to higher levels of expression. Very high levels of expression might overload the capacity of the mammary gland to perform posttranslation modifications, and secretion of lysosomal proteins. However, the data presented below indicate that substantial posttranslational modification occurs including the formation of mannose 6-phosphate groups, notwithstanding a high expression level in the mg/ml range. Substantial modification means that at least about 10, 25, 50, 75 or 90% of secreted molecules bear at least one mannose 6-phosphate group. Thus, genomic constructs or hybrid cDNA-genomic constructs are generally preferred.

In genomic constructs, it is not necessary to retain all intronic sequences. For example, some intronic sequences can be removed to obtain a smaller transgene facilitating DNA manipulations and subsequent microinjection. See Archibald et al., WO 90/05188 (incorporated by reference in its entirety for all purposes). Removal of some introns is also useful in some instances to reduce expression levels and thereby ensure that posttranslational modification is substantially complete. In other instances excluding an intron such as intron one from the genomic sequence of acid α-glucosidase leads to a higher expression of the mature enzyme. It is also possible to delete some or all of noncoding exons. In some transgenes, selected nucleotides in lysosomal protein encoding sequences are mutated to remove proteolytic cleavage sites.

Because the intended use of lysosomal proteins produced by transgenic mammals is usually administration to humans, the species from which the DNA segment encoding a lysosomal protein sequence is obtained is preferably human. Analogously if the intended use were in veterinary therapy (e.g., on a horse, dog or cat), it is preferable that the DNA segment be from the same species.

The promoter and enhancer are from a gene that is exclusively or at least preferentially expressed in the mammary gland (i.e., a mammary-gland specific gene). Preferred genes as a source of promoter and enhancer include β-casein, κ-casein, αS1-casein, αS2-casein, β-lactoglobulin, whey acid protein, and α-lactalbumin. The promoter and enhancer

are usually but not always obtained from the same mammary-gland specific gene. This gene is sometimes but not necessarily from the same species of mammal as the mammal into which the transgene is to be expressed. Expression regulation sequences from other species such as those from human genes can also be used. The signal sequence must be capable of directing the secretion of the lysosomal protein from the mammary gland. Suitable signal sequences can be derived from mammalian genes encoding a secreted protein. Surprisingly, the natural signal sequences of lysosomal proteins are suitable, notwithstanding that these proteins are normally not secreted but targeted to an intracellular organelle. In addition to such signal sequences, preferred sources of signal sequences are the signal sequence from the same gene as the promoter and enhancer are obtained. Optionally, additional regulatory sequences are included in the transgene to optimize expression levels. Such sequences include 5' flanking regions, 5' transcribed but untranslated regions, intronic sequences, 3' transcribed but untranslated regions, polyadenylation sites, and 3' flanking regions. Such sequences are usually obtained either from the mammary-gland specific gene from which the promoter and enhancer are obtained or from the lysosomal protein gene being expressed. Inclusion of such sequences produces a genetic milieu simulating that of an authentic mammary gland specific gene and/or that of an authentic lysosomal protein gene. This genetic milieu results in some cases (e.g., bovine αS1-casein) in higher expression of the transcribed gene. Alternatively, 3' flanking regions and untranslated regions are obtained from other heterologous genes such as the P-globin gene or viral genes. The inclusion of 3' and 5' untranslated regions from a lysosomal protein gene, or other heterologous gene can also increase the stability of the transcript.

In some embodiments, about 0.5, 1, 5, 10, 15, 20 or 30 kb of 5' flanking sequence is included from a mammary specific gene in combination with about 1, 5, 10, 15, 20 or 30 kb or 3' flanking sequence from the lysosomal protein gene being expressed. If the protein is expressed from a cDNA sequence, it is advantageous to include an intronic sequence between the promoter and the coding sequence. The intronic sequence is preferably a hybrid sequence formed from a 5' portion from an intervening sequence from the first intron of the mammary gland specific region from which the promoter is obtained and a 3' portion from an intervening sequence of an IgG intervening sequence or lysosomal protein gene. See DeBoer et al., WO 91/08216 (incorporated by reference in its entirety for all purposes).

A preferred transgene for expressing a lysosomal protein comprises a cDNA-genomic hybrid lysosomal protein gene-linked 5' to a casein promoter and enhancer. The hybrid gene includes the signal sequence, coding region, and a 3' flanking region from the lysosomal protein gene. Optionally, the cDNA segment includes an intronic sequence between the 5' casein and untranslated region of the gene encoding the lysosomal protein. Of course, corresponding cDNA and genomic segments can also be fused at other locations within the gene provided a contiguous protein can be expressed from the resulting fusion.

Other preferred transgenes have a genomic lysosomal protein segment linked 5' to casein regulatory sequences. The genomic segment is usually contiguous from the 5' untranslated region to the 3' flanking region of the gene. Thus, the genomic segment includes a portion of the lysosomal protein 5' untranslated sequence, the signal sequence, alternating introns and coding exons, a 3' untranslated region, and a 3' flanking region. The genomic segment is linked via the 5' untranslated region to a casein fragment comprising a promoter and enhancer and usually a 5' untranslated region.

DNA sequence information is available for all of the mammary gland specific genes listed above, in at least one, and often several organisms. See, e.g., Richards et al., J. Biol. Chem. 256, 526-532 (1981) (α-lactalbumin rat); Campbell et al., Nucleic Acids Res. 12, 8685-8697 (1984) (rat WAP); Jones et al., J. Biol. Chem. 260, 7042-7050 (1985)) (rat. β-casein); Yu-Lee and Rosen, J. Biol. Chem. 258, 10794-10804 (1983) (rat γ casein)); Hall, Biochem. J. 242, 735-742 (1987) (α-lactalbumin human); Stewart, Nucleic Acids Res. 12, 389 (1984) (bovine αs1 and K casein cDNAs); Gorodetsky et al., Gene 66, 87-96 (1988) (bovine β casein); Alexander et al., Eur. J. Biochem. 178, 395-401 (1988) (bovine κ casein); Brignon et al., FEBS Lett. 188, 48-55 (1977) (bovine αS2 casein); Jamieson et al., Gene 61, 85-90 (1987), Ivanov et al., Biol. Chem. Hoppe-Seyler 369, 425-429 (1988), Alexander et al., Nucleic Acids Res. 17, 6739 (1989) (bovine β lactoglobulin); Vilotte et al., Biochimie 69, 609-620 (1987) (bovine α-lactalbumin) (incorporated by reference in their entirety for all purposes). The structure and function of the various milk protein genes are reviewed by Mercier and Vilotte, J. Dairy Sci. 76, 3079-3098 (1993) (incorporated by reference in its entirety for all purposes). To the extent that additional sequence data might be required, sequences flanking the regions already obtained could be readily cloned using the existing sequences as probes. Mammary-gland specific regulatory sequences from different organisms are likewise obtained by screening libraries from such organisms using known cognate nucleotide sequences, or antibodies to cognate proteins as probes.

General strategies and exemplary transgenes employing αS1-casein regulatory sequences for targeting the expression of a recombinant protein to the mammary gland are described in more detail in DeBoer et al., WO 91/08216 and WO 93/25567 (incorporated by reference in their entirety for all purposes). Examples of transgenes employing regulatory sequences from other mammary gland specific genes have also been described. See, e.g., Simon et al., Bio/Technology 6, 179-183 (1988) and WO88/00239 (1988) (β-lactoglobulin regulatory sequence for expression in sheep); Rosen, EP 279, 582 and Lee et al., Nucleic Acids Res. 16, 1027-1041 (1988) (β-casein regulatory sequence for expression in mice); Gordon, Biotechnology 5, 1183 (1987) (WAP regulatory sequence for expression in mice); WO 88/01648 (1988) and Eur. J. Biochem. 186, 43-48 (1989) (α-lactalbumin regulatory sequence for expression in mice) (incorporated by reference in their entirety for all purposes).

The expression of lysosomal proteins in the milk from transgenes can be influenced by co-expression or functional inactivation (i.e., knock-out) of genes involved in post translational modification and targeting of the lysosomal proteins. The data in the Examples indicate that surprisingly mammary glands already express modifying enzymes at sufficient quantities to obtain assembly and secretion of mannose 6-phosphate containing proteins at high levels. However, in some transgenic mammals expressing these proteins at high levels, it is sometimes preferable to supplement endogenous levels of processing enzymes with additional enzyme resulting from transgene expression. Such transgenes are constructed employing similar principles to those discussed above with the processing enzyme coding sequence replacing the lysosomal protein coding sequence in the transgene. It is not generally necessary that posttranslational processing enzymes be secreted. Thus, the secretion signal sequence linked to the lysosomal protein coding sequence is replaced with a signal sequence that targets the processing enzyme to

the endoplasmic reticulum without secretion. For example, the signal sequences naturally associated with these enzymes are suitable.

D. Transgenesis

The transgenes described above are introduced into nonhuman mammals. Most nonhuman mammals, including rodents such as mice and rats, rabbits, ovines such as sheep and goats, porcines such as pigs, and bovines such as cattle and buffalo, are suitable. Bovines offer an advantage of large yields of milk, whereas mice offer advantages of ease of transgenesis and breeding. Rabbits offer a compromise of these advantages. A rabbit can yield 100 ml milk per day with a protein content of about 14% (see Buhler et al., Biotechnology 8, 140 (1990)) (incorporated by reference in its entirety for all purposes), and yet can be manipulated and bred using the same principles and with similar facility as mice. Nonviviparous mammals such as a spiny anteater or duckbill platypus are typically not employed.

In some methods of transgenesis, transgenes are introduced into the pronuclei of fertilized oocytes. For some animals, such as mice and rabbits, fertilization is performed in vivo and fertilized ova are surgically removed. In other animals, particularly bovines, it is preferable to remove ova from live or slaughterhouse animals and fertilize the ova in vitro. See DeBoer et al., WO 91/08216. In vitro fertilization permits a transgene to be introduced into substantially synchronous cells at an optimal phase of the cell cycle for integration (not later than S-phase). Transgenes are usually introduced by microinjection. See U.S. Pat. No. 4,873,292. Fertilized oocytes are then cultured in vitro until a pre-implantation embryo is obtained containing about 16-150 cells. The 16-32 cell stage of an embryo is described as a morula. Pre-implantation embryos containing more than 32 cells are termed blastocysts. These embryos show the development of a blastocoele cavity, typically at the 64 cell stage. Methods for culturing fertilized oocytes to the pre-implantation stage are described by Gordon et al., Methods Enzymol. 101, 414 (1984); Hogan et al., Manipulation of the Mouse Embryo: A Laboratory Manual, C.S.H.L. N.Y. (1986) (mouse embryo); and Hammer et al., Nature 315, 680 (1985) (rabbit and porcine embryos); Gandolfi et al. J. Reprod. Fert. 81, 23-28 (1987); Rexroad et al., J. Anim. Sci. 66, 947-953 (1988) (ovine embryos) and Eyestone et al. J. Reprod. Fert. 85, 715-720 (1989); Camous et al., J. Reprod. Fert. 72, 779-785 (1984); and Heyman et al. Theriogenology 27, 5968 (1987) (bovine embryos) (incorporated by reference in their entirety for all purposes). Sometimes pre-implantation embryos are stored frozen for a period pending implantation. Pre-implantation embryos are transferred to the oviduct of a pseudopregnant female resulting in the birth of a transgenic or chimeric animal depending upon the stage of development when the transgene is integrated. Chimeric mammals can be bred to form true germline transgenic animals.

Alternatively, transgenes can be introduced into embryonic stem cells (ES). These cells are obtained from preimplantation embryos cultured in vitro. Bradley et al., Nature 309, 255-258 (1984) (incorporated by reference in its entirety for all purposes). Transgenes can be introduced into such cells by electroporation or microinjection. Transformed ES cells are combined with blastocysts from a non-human animal. The ES cells colonize the embryo and in some embryos form the germline of the resulting chimeric animal. See Jaenisch, Science, 240, 1468-1474 (1988) (incorporated by reference in its entirety for all purposes). Alternatively, ES cells can be used as a source of nuclei for transplantation into an enucleated fertilized oocyte giving rise to a transgenic mammal.

For production of transgenic animals containing two or more transgenes, the transgenes can be introduced simultaneously using the same procedure as for a single transgene. Alternatively, the transgenes can be initially introduced into separate animals and then combined into the same genome by breeding the animals. Alternatively, a first transgenic animal is produced containing one of the transgenes. A second transgene is then introduced into fertilized ova or embryonic stem cells from that animal. In some embodiments, transgenes whose length would otherwise exceed about 50 kb, are constructed as overlapping fragments. Such overlapping fragments are introduced into a fertilized oocyte or embryonic stem cell simultaneously and undergo homologous recombination in vivo. See Kay et al., WO 92/03917 (incorporated by reference in its entirety for all purposes).

E. Characteristics of Transgenic Mammals

Transgenic mammals of the invention incorporate at least one transgene in their genome as described above. The transgene targets expression of a DNA segment encoding a lysosomal protein at least predominantly to the mammary gland. Surprisingly, the mammary glands are capable of expressing proteins required for authentic posttranslation processing including steps of oligosaccharide addition and phosphorylation. Processing by enzymes in the mammary gland results in phosphorylation of the 6' position of mannose groups.

Lysosomal proteins can be secreted at high levels of at least 10, 50, 100, 500, 1000, 2000, 5000 or 10,000 .μg/ml. Surprisingly, the transgenic mammals of the invention exhibit substantially normal health. Secondary expression of lysosomal proteins in tissues other than the mammary gland does not occur to an extent sufficient to cause deleterious effects. Moreover, exogenous lysosomal protein produced in the mammary gland is secreted with sufficient efficiency that no significant problem is presented by deposits clogging the secretory apparatus.

The age at which transgenic mammals can begin producing milk, of course, varies with the nature of the animal. For transgenic bovines, the age is about two-and-a-half years naturally or six months with hormonal stimulation, whereas for transgenic mice the age is about 5-6 weeks. Of course, only the female members of a species are useful for producing milk. However, transgenic males are also of value for breeding female descendants. The sperm from transgenic males can be stored frozen for subsequent in vitro fertilization and generation of female offspring

F. Recovery of Proteins from Milk

Transgenic adult female mammals produce milk containing high concentrations of exogenous lysosomal protein. The protein can be purified from milk, if desired, by virtue of its distinguishing physical and chemical properties, and standard purification procedures such as precipitation, ion exchange, molecular exclusion or affinity chromatography. See generally Scopes, Protein Purification (Springer-Verlag, N.Y., 1982).

Purification of human acid α-glucosidase from milk can be carried out by defatting of the transgenic milk by centrifugation and removal of the fat, followed by removal of caseins by high speed centrifugation followed by dead-end filtration (i.e., dead-end filtration by using successively declining filter sizes) or cross-flow filtration, or; removal of caseins directly by cross-flow filtration. Human acid α-glucosidase is purified by chromatography, including Q Sepharose FF (or other anion-exchange matrix), hydrophobic interaction chromatography (HIC), metal-chelating Sepharose, or lectins coupled to Sepharose (or other matrices).

Q Sepharose Fast Flow chromatography may be used to purify human acid α-glucosidase present in filtered whey or whey fraction as follows: a Q Sepharose Fast Flow (QFF; Pharmacia) chromatography (Pharmacia XK-50 column, 15 cm bed height; 250 cm/hr flow rate) the column was equilibrated in 20 mM sodium phosphate buffer, pH 7.0 (buffer A); the S/D-incubated whey fraction (about 500 to 600 ml) is loaded and the column is washed with 4-6 column volumes (cv) of buffer A (20 mM sodium phosphate buffer, pH 7.0). The human acid α-glucosidase fraction is eluted from the Q FF column with 2-3 cv buffer A, containing 100 mM NaCl.

The Q FF Sepharose human acid α-glucosidase containing fraction can be further purified using Phenyl Sepharose High Performance chromatography. For example, 1 vol. of 1 M ammonium sulphate is added to the Q FF Sepharose human acid α-glucosidase eluate while stirring continuously. Phenyl HP (Pharmacia) column chromatography (Pharmacia XK-50 column, 15 cm bed height; 150 cm/hr flow rate) is then done at room temperature by equilibrating the column in 0.5 M ammonium sulphate, 50 mM sodium phosphate buffer pH 6.0 (buffer C), loading the 0.5 M ammoniumsulphate-incubated human acid α-glucosidase eluate (from Q FF Sepharose), washing the column with 24 cv of buffer C, and eluting the human acid α-glucosidase was eluted from the Phenyl HP column with 3-5 cv buffer D (50 mM sodium phosphate buffer at pH 6.0). Alternative methods and additional methods for further purifying human acid α-glucosidase will be apparent to those of skill. For example, see United Kingdom patent application 998 07464.4 (incorporated by reference in its entirety for all purposes).

G. Uses of Recombinant Lysosomal Proteins

The recombinant lysosomal proteins produced according to the invention find use in enzyme replacement therapeutic procedures. A patient having a genetic or other deficiency resulting in an insufficiency of functional lysosomal enzyme can be treated by administering exogenous enzyme to the patient. Patients in need of such treatment can be identified from symptoms (e.g., Hurler's syndrome symptoms include Dwarfism, corneal clouding, hepatosplenomegaly, valvular lesions, coronary artery lesions, skeletal deformities, joint stiffness and progressive mental retardation). Alternatively, or additionally, patients can be diagnosed from biochemical analysis of a tissue sample to reveal excessive accumulation of a characteristic metabolite processed by a particular lysosomal enzyme or by enzyme assay using an artificial or natural substrate to reveal deficiency of a particular lysosomal enzyme activity. For most diseases, diagnosis can be made by measuring the particular enzyme deficiency or by DNA analysis before occurrence of symptoms or excessive accumulation of metabolites (Scriver et al., supra, chapters on lysosomal storage disorders). All of the lysosomal storage diseases are hereditary. Thus, in offspring from families known to have members suffering from lysosomal diseases, it is sometimes advisable to commence prophylactic treatment even before a definitive diagnosis can be made.

Pharmaceutical Compositions

In some methods, lysosomal enzymes are administered in purified form together with a pharmaceutical carrier as a pharmaceutical composition. The preferred form depends on the intended mode of administration and therapeutic application. The pharmaceutical carrier can be any compatible, nontoxic substance suitable to deliver the polypeptides to the patient. Sterile water, alcohol, fats, waxes, and inert solids may be used as the carrier. Pharmaceutically-acceptable adjuvants, buffering agents, dispersing agents, and the like, may also be incorporated into the pharmaceutical compositions.

The concentration of the enzyme in the pharmaceutical composition can vary widely, i.e., from less than about 0.1% by weight, usually being at least about 1% by weight to as much as 20% by weight or more.

For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. Active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

A typical composition for intravenous infusion could be made up to contain 100 to 500 ml of sterile 0.9% NaCl or 5% glucose optionally supplemented with a 20% albumin solution and 100 to 500 mg of an enzyme. A typical pharmaceutical compositions for intramuscular injection would be made up to contain, for example, 1 ml of sterile buffered water and 1 to 10 mg of the purified alpha glucosidase of the present invention. Methods for preparing parenterally administrable compositions are well known in the art and described in more detail in various sources, including, for example, Remington's Pharmaceutical Science (15th ed., Mack Publishing, Easton, Pa., 1980) (incorporated by reference in its entirety for all purposes).

AGLU can be formulated in 10 mM sodium phosphate buffer pH 7.0. Small amounts of ammonium sulphate are optionally present (<10 mM). The enzyme is typically kept frozen at about −70.degree. C., and thawed before use. Alternatively, the enzyme may be stored cold (e.g., at about 4.degree. C. to 8.degree. C.) in solution. In some embodiments, AGLU solutions comprise a buffer (e.g., sodium phosphate, potassium phosphate or other physiologically acceptable buffers), a simple carbohydrate (e.g., sucrose, glucose, maltose, mannitol or the like), proteins (e.g., human serum albumin), and/or surfactants (e.g., polysorbate 80 (Tween-80), cremophore-EL, cremophore-R, labrofil, and the like).

AGLU can also be stored in lyophilized form. For lyophilization, AGLU can be formulated in a solution containing mannitol, and sucrose in a phosphate buffer. The concentration of sucrose should be sufficient to prevent aggregation of AGLU on reconstitution. The concentration of mannitol should be sufficient to significantly reduce the time otherwise needed for lyophilization. The concentrations of mannitol and sucrose should, however, be insufficient to cause unacceptable hypertonicity on reconstitution. Concentrations of mannitol and sucrose of 1-3 mg/ml and 0.1-1.0 mg/ml respectively are suitable. Preferred concentrations are 2 mg/ml mannitol and 0.5 mg/ml sucrose. AGLU is preferably at 5 mg/ml before lyophilization and after reconstitution. Saline preferably at 0.9% is a preferred solution for reconstitution.

For AGLU purified from rabbit milk, a small amount of impurities (e.g., up to about 5%/o) can be tolerated. Possible

impurities may be present in the form of rabbit whey proteins. Other possible impurities are structural analogues (e.g., oligomers and aggregates) and truncations of AGLU. Current batches indicate that the AGLU produced in transgenic rabbits is >95% pure. The largest impurities are rabbit whey proteins, although on gel electrophoresis, AGLU bands of differing molecular weights are also seen.

Infusion solutions should be prepared aseptically in a laminar air flow hood. The appropriate amount of AGLU should be removed from the freezer and thawed at room temperature. Infusion solutions can be prepared in glass infusion bottles by mixing the appropriate amount of AGLU finished product solution with an adequate amount of a solution containing human serum albumin (HSA) and glucose. The final concentrations can be 1% HSA and 4% glucose for 25-200 mg doses and 1% HSA and 4% glucose for 400-800 mg doses. HSA and AGLU can be filtered with a 0.2 .μm syringe filter before transfer into the infusion bottle containing 5% glucose. Alternatively, AGLU can be reconstituted in saline solution, preferably 0.9% for infusion. Solutions of AGLU for infusion have been shown to be stable for up to 7 hours at room temperature. Therefore the AGLU solution is preferably infused within seven hours of preparation.

Therapeutic Methods

The present invention provides effective methods of treating Pompe's disease. These methods are premised in part on the availability of large amounts of human acid alpha glucosidase in a form that is catalytically active and in a form that can be taken up by tissues, particularly, liver, heart and muscle (e.g., smooth muscle, striated muscle, and cardiac muscle), of a patient being treated. Such human acid alpha-glucosidase is provided from e.g., the transgenic animals described in the Examples. The alpha-glucosidase is preferably predominantly (i.e., >50%) in the precursor form of about 100-110 kD. (The apparent molecular weight or relative mobility of the 100-110 kD precursor may vary somewhat depending on the method of analysis used, but is typically within the range 95 kD and 120 kD.) Given the successful results with human acid alpha-glucosidase in the transgenic animals discussed in the Examples, it is possible that other sources of human alpha-glucosidase, such as resulting from cellular expression systems, can also be used. For example, an alternative way to produce human acid α-glucosidase is to transfect the acid α-glucosidase gene into a stable eukaryotic cell line (e.g., CHO) as a cDNA or genomic construct operably linked to a suitable promoter. However, it is more laborious to produce the large amounts of human acid alpha glucosidase needed for clinical therapy by such an approach.

The pharmaceutical compositions of the present invention are usually administered intravenously. Intradermal, intramuscular or oral administration is also possible in some circumstances. The compositions can be administered for prophylactic treatment of individuals suffering from, or at risk of, a lysosomal enzyme deficiency disease. For therapeutic applications, the pharmaceutical compositions are administered to a patient suffering from established disease in an amount sufficient to reduce the concentration of accumulated metabolite and/or prevent or arrest further accumulation of metabolite. For individuals at risk of lysosomal enzyme deficiency disease, the pharmaceutical compositions are administered prophylactically in an amount sufficient to either prevent or inhibit accumulation of metabolite. An amount adequate to accomplish this is defined as a "therapeutically-" or "prophylactically-effective dose." Such effective dosages will depend on the severity of the condition and on the general state of the patient's health.

In the present methods, human acid alpha glucosidase is usually administered at a dosage of 10 mg/kg patient body weight or more per week to a patient. Often dosages are greater than 10 mg/kg per week. Dosages regimes can range from 10 mg/kg per week to at least 1000 mg/kg per week. Typically dosage regimes are 10 mg/kg per week, 15 mg/kg per week, 20 mg/kg per week, 25 mg/kg per week, 30 mg/kg per week, 35 mg/kg per week, 40 mg/kg week, 45 mg/kg per week, 60 mg/kg week, 80 mg/kg per week and 120 mg/kg per week. In preferred regimes 10 mg/kg, 15 mg/kg, 20 mg/kg, 30 mg/kg or 40 mg/kg is administered once, twice or three times weekly. Treatment is typically continued for at least 4 weeks, sometimes 24 weeks, and sometimes for the life of the patient. Treatment is preferably administered i.v. Optionally, levels of human alpha-glucosidase are monitored following treatment (e.g., in the plasma or muscle) and a further dosage is administered when detected levels fall substantially below (e.g., less than 20%) of values in normal persons.

In some methods, human acid alpha glucosidase is administered at an initially "high" dose (i.e., a "loading dose"), followed by administration of a lower doses (i.e., a "maintenance dose"). An example of a loading dose is at least about 40 mg/kg patient body weight 1 to 3 times per week (e.g., for 1, 2, or 3 weeks). An example of a maintenance dose is at least about 5 to at least about 10 mg/kg patient body weight per week, or more, such as 20 mg/kg per week, 30 mg/kg per week, 40 mg/kg week.

In some methods, a dosage is administered at increasing rate during the dosage period. Such can be achieved by increasing the rate of flow intravenous infusion or by using a gradient of increasing concentration of alpha-glucosidase administered at constant rate. Administration in this manner reduces the risk of immunogenic reaction. In some dosages, the rate of administration measured in units of alpha glucosidase per unit time increases by at least a factor of ten. Typically, the intravenous infusion occurs over a period of several hours (e.g., 1-10 hours and preferably 2-8 hours, more preferably 3-6 hours), and the rate of infusion is increased at intervals during the period of administration.

Suitable dosages (all in mg/kg/hr) for infusion at increasing rates are shown in table 1 below. The first column of the table indicates periods of time in the dosing schedule. For example, the reference to 0-1 hr refers to the first hour of the dosing. The fifth column of the table shows the range of doses than can be used at each time period. The fourth column shows a narrower included range of preferred dosages. The third column indicates upper and lower values of dosages administered in an exemplary clinical trial. The second column shows particularly preferred dosages, these representing the mean of the range shown in the third column of table 1.

TABLE 1

Time	Mean Doses (I)	Lower&Upper Values	Preferred Range	Range
0-1 hr:	0.3 mg/kg/hr	0.25-0.4	0.1-1	0.03-3
1-2 hr:	1 mg/kg/hr	0.9-1.4	1-4	0.3-12
2.2.5 hr:	4 mg/kg/hr	3.6-5.7	3-10	1-30
2.5-5.6 hr:	12 mg/kg/hr	7.2-11.3	6-20	2.60

The methods are effective on patients with both early onset (infantile) and late onset (juvenile and adult) Pompe's disease. In patients with the infantile form of Pompe's disease symptoms become apparent within the first 4 months of life. Mostly, poor motor development and failure to thrive are noticed first. On clinical examination, there is generalized hypotonia with muscle wasting, increased respiration rate

with sternal retractions, moderate enlargement of the liver, and protrusion of the tongue. Ultrasound examination of the heart shows a progressive hypertrophic cardiomyopathy, eventually leading to insufficient cardiac output. The ECG is characterized by marked left axis deviation, a short PR interval, large QRS complexes, inverted T waves and ST depression. The disease shows a rapidly progressive course leading to cardiorespiratory failure within the first year of life. On histological examination at autopsy lysosomal glycogen storage is observed in various tissues, and is most pronounced in heart and skeletal muscle. Treatment with human acid alpha glucosidase in the present methods results in a prolongation of life of such patients (e.g., greater than 1, 2, 5 years up to a normal lifespan). Treatment can also result in elimination or reduction of clinical and biochemical characteristics of Pompe's disease as discussed above. Treatment is administered soon after birth, or antenatally if the parents are known to bear variant alpha glucosidase alleles placing their progeny at risk.

Patients with the late onset adult form of Pompe's disease may not experience symptoms within the first two decades of life. In this clinical subtype, predominantly skeletal muscles are involved with predilection of those of the limb girdle, the trunk and the diaphragm. Difficulty in climbing stairs is often the initial complaint. The respiratory impairment varies considerably. It can dominate the clinical picture, or it is not experienced by the patient until late in life. Most such patients die because of respiratory insufficiency. In patients with the juvenile subtype, symptoms usually become apparent in the first decade of life. As in adult Pompe's disease, skeletal muscle weakness is the major problem; cardiomegaly, hepatomegaly, and macroglossia can be seen, but are rare. In many cases, nightly ventilatory support is ultimately needed. Pulmonary infections in combination with wasting of the respiratory muscles are life threatening and mostly become fatal before the third decade. Treatment with the present methods prolongs the life of patients with late onset juvenile or adult Pompe's disease up to a normal life span. Treatment also eliminates or significantly reduces clinical and biochemical symptoms of disease.

Other Uses

Lysosomal proteins produced in the milk of transgenic animals have a number of other uses. For example, α-glucosidase, in common with other α-amylases, is an important tool in production of starch, beer and pharmaceuticals. See. Vihinen and Mantsala, Crit. Rev. Biochem. Mol. Biol. 24, 329-401 (1989) (incorporated by reference in its entirety for all purpose). Lysosomal proteins are also useful for producing laboratory chemicals or food products. For example, acid α-glucosidase degrades 1,4 and 1,6 α-glucidic bonds and can be used for the degradation of various carbohydrates containing these bonds, such as maltose, isomaltose, starch and glycogen, to yield glucose. Acid α-glucosidase is also useful for administration to patients with an intestinal maltase or isomaltase deficiency. Symptoms otherwise resulting from the presence of undigested maltose are avoided. In such applications, the enzyme can be administered without prior fractionation from milk, as a food product derived from such milk (e.g., ice cream or cheese) or as a pharmaceutical composition. Purified recombinant lysosomal enzymes are also useful for inclusion as controls in diagnostic kits for assay of unknown quantities of such enzymes in tissue samples.

EXAMPLES

Example 1

Construction of Transgenes

(a) cDNA Construct

Construction of an expression vector containing cDNA encoding human acid α-glucosidase started with the plasmid p16,8hlf3 (see DeBoer et al. (1991) and (1993), supra) This plasmid includes bovine αS1-casein regulatory sequences. The lactoferrin cDNA insert of the parent plasmid was exchanged for the human acid α-glucosidase cDNA (Hoefsloot et al. EMBO J. 7, 1697-1704 (1988)) at the ClaI site and SalI site of the expression cassette as shown in FIG. **1**. To obtain the compatible restriction sites at the ends of the α-glucosidase cDNA fragment, plasmid pSHAG2 (id.) containing the complete cDNA encoding human α-glucosidase was digested with EcoRI and SphI and the 3.3 kb cDNA-fragment was subcloned in pKUN7ΔC, a pKUN1 derivative (Konings et al., Gene 46, 269-276 (1986)), with a destroyed ClaI site within the vector nucleotide sequences and with a newly designed polylinker: HindIII ClaI EcoRI SphI XhoI EcoRI SfiI SfiI/SmaI NotI EcoRI* (*=destroyed site). From the resulting plasmid pagluCESX, the 3.3-kb cDNA-fragment could be excised by ClaI and XhoI. This fragment was inserted into the expression cassette shown in FIG. **1** at the ClaI site and XhoI-compatible SalI site. As a result, the expression plasmid p16,8αglu consists of the cDNA sequence encoding human acid α-glucosidase flanked by bovine αS1 casein sequences as shown in FIG. **1**. The 27.3-kb fragment containing the complete expression cassette can be excised by cleavage with NotI (see FIG. **1**).

(b) Genomic Constructs

Construct c8αgluex1 contains the human acid α-glucosidase gene (Hoefsloot et al., Biochem. J. 272, 493-497 (1990)); starting in exon 1 just downstream of its transcription initiation site (see FIG. **2**, panel A). Therefore, the construct encodes almost a complete 5' UTR of the human acid α-glucosidase gene. This fragment was fused to the promoter sequences of the bovine αS1-casein gene. The αS1-casein initiation site is present 22 bp upstream of the αS1-casein/acid α-glucosidase junction. The construct has the human acid α-glucosidase polyadenylation signal and 3' flanking sequences. Construct c8αgluex2 contains the bovine αS1-casein promoter immediately fused to the translation initiation site in exon 2 of the human acid α-glucosidase gene (see FIG. **2**, panel B). Thus, the αS1-casein transcription initiation site and the α-glucosidase translation initiation site are 22-bp apart in this construct. Therefore no α-glucosidase 5' UTR is preserved. This construct also contains the human acid α-glucosidase polyadenylation signal and 3' flanking sequences as shown in FIG. **2**, panel B.

Construct c8,8αgluex2-20 differs from construct c8αgluex2 only in the 3' region. A SphI site in exon 20 was used to fuse the bovine αS1-casein 3' sequence to the human acid α-glucosidase construct. The polyadenylation signal is located in this 3' αS1-casein sequence (FIG. **2**, panel C).

Construct c8,8αgluex2-20 differs from construct c8αgluex2 only in the 3' region. A SphI site in exon 20 was used to fuse the bovine αS1-casein 3' sequence to the human acid α-glucosidase construct. The polyadenylation signal is located in this 3' αS1 casein sequence (FIG. **2**, panel C).

Methods for Construction of Genomic Constructs

Three contiguous BglII fragments containing the human acid α-glucosidase gene were isolated by Hoefsloot et al.,

supra. These fragments were ligated in the BglII-site of pKUN8ΔC, a pKUN7ΔC derivative with a customized polylinker: HindIII ClaI StuI SstI BglII PvnI NcoI EcoRI SphI XhoI EcoRI* SmaI/SfiI NotI EcoRI* (*=destroyed site). This ligation resulted in two orientations of the fragments generating plasmids p7.3αgluBES, p7.3αgluBSE, p8.5αgluBSE, p8.5αgluBES, p10αgluBSE and p10αgluBES.

Because unique NotI-sites at the ends of the expression cassette are used subsequently for the isolation of the fragments used for microinjection, the intragenic NotI site in intron 17 of human acid α-glucosidase had to be destroyed. Therefore, p10αgluBES was digested with ClaI and XhoI; the fragment containing the 3' α-glucosidase end was isolated. This fragment was inserted in the ClaI and XhoI sites of pKUN10ΔC, resulting in p10αgluNV. Previously pKUN10ΔC (i.e., a derivative of pKUN8ΔC) was obtained by digesting pKUN8ΔC with NotI, filling in the sticky ends with Klenow and subsequently, annealing the plasmid by blunt-ended ligation. Finally, p10αgluΔNV was digested with NotI. These sticky ends were also filled with Klenow and the fragment was ligated, resulting in plasmid p10αgluΔNotI.

Construction of c8αgluex1

Since the SstI site in first exon of the α-glucosidase gene was chosen for the fusion to the bovine αS1-casein sequence, p8.5αgluBSE was digested with BglII followed by a partial digestion with SstI. The fragment containing exon 1-3 was isolated and ligated into the BglII and SstI sites of pKUN8ΔC. The resulting plasmid was named: p5'αgluex1. (see FIG. **3**, panel A).

The next step (FIG. **3**, panel B) was the ligation of the 3' part to p5'αgluex1. First, p10αgluΔN was digested with BglII and BamHI. This fragment containing exon 16-20 was isolated. Second, p5'αgluex1 was digested with BglII and to prevent self-ligation, and treated with phosphorylase (BAP) to dephosphorylate the sticky BglII ends. Since BamHI sticky ends are compatible with the BglII sticky ends, these ends ligate to each other. The resulting plasmid, i.e., p5'3'αgluex1, was selected. This plasmid has a unique BglII site available for the final construction step (see FIG. **3**, panels B and C).

The middle part of the α-glucosidase gene was inserted into the latter construct. For this step, p7.3αgluBSE was digested with BglII, the 8.5-kb fragment was isolated and ligated to the BglII digested and dephosphorylated p5'3'αgluex1 plasmid. The resulting plasmid is pαgluex1 (FIG. **3**, panel C).

The bovine αS1-casein promoter sequences were incorporated in the next step via a ligation involving three fragments. The pWE15 cosmid vector was digested with NotI and dephosphorylated. The bovine αS1-casein promoter was isolated as an 8 Rb NotI-ClaI fragment (see de Boer et al., 1991, supra). The human acid α-glucosidase fragment was isolated from pαgluex1 using the same enzymes. These three fragments were ligated and packaged using the Stratagene GigapackII kit in 1046 *E. coli* host cells. The resulting cosmid c8αgluex1 was propagated in *E. coli* strain DH5α. The vector was linearized with NotI before microinjection.

Construction of c8αgluex2 and c8.8αgluex2-20

The construction of the other two expression plasmids (FIG. **2**, panels B and C) followed a similar strategy to that of c8αgluex1. The plasmid p5'αgluStuI was derived from p8,5αgluBSE by digestion of the plasmid with StuI, followed by self-ligation of the isolated fragment containing exon 2-3 plus the vector sequences. Plasmid p5'αgluStuI was digested with PglII followed by a partial digestion of the linear fragment with NcoI resulting in several fragments. The 2.4 kb fragment, containing exon 2 and 3, was isolated and ligated into the NcoI and BglII sites of vector pKUN12ΔC, resulting in p5'αgluex2. Note that pKUN12ΔC is a derivative of pKUN8ΔC containing the polylinker: ClaI NcoI BglII HindiI EcoRI SphI XhoI SmaI/SfiI NotI.

The plasmid p10αgluΔNotI was digested with BglII and HindIII. The fragment containing exons 16-20 was isolated and ligated in p5'αgluex2 digested with BglII and HindIII. The resulting plasmid was p5'3'αgluex2. The middle fragment in p5'3'αgluex2 was inserted as for pαgluex1. For this, p7.3αglu was digested with BglII. The fragment was isolated and ligated to the BglII-digested and dephosphorylated p5'3'αgluex2. The resulting plasmid, pαgluex2, was used in construction of c8αgluex-20 and c8,8αgluex2-20 (FIG. **2**, panels B and C).

For the construction of third expression plasmid c8,8α gluex2-20 (FIG. **2**, panel C) the 3' flanking region of α-glucosidase was deleted. To achieve this, pαgluex2 was digested with SphI. The fragment containing exon 2-20 was isolated and self-ligated resulting in pαgluex2-20. Subsequently, the fragment containing the 3' flanking region of bovine αS1-casein gene was isolated from p16,8αglu by digestion with SphI and NotI. This fragment was inserted into pαgluex2-20 using the SpII site and the NotI site in the polylinker sequence resulting in pαgluex2-20-3αS1.

The final step in generating c8,8αgluex2-20 was the ligation of three fragments as in the final step in the construction leading to c8αgluex1. Since the ClaI site in pαgluex2-20-3'αS1 and pαgluex2 appeared to be uncleavable due to methylation, the plasmids had to be propagated in the *E. coli* DAM(−) strain ECO343. The pαgluex2-20-3'αS1 isolated from that strain was digested with ClaI and NotI. The fragment containing exons 220 plus the 3' αS1-casein flanking region was purified from the vector sequences. This fragment, an 8 kb NotI-ClaI fragment containing the bovine αS1 promoter (see DeBoer (1991) and (1993), supra) and NotI-digested, dephosphorylated pWE15 were ligated and packaged. The resulting cosmid is c8,8αgluex2-20.

Cosmid c8αgluex2 (FIG. **2**, panel B) was constructed via a couple of different steps. First, cosmid c8,8αgluex2-20 was digested with SalI and NotI. The 10.5-kb fragment containing the αS1-promoter and the exons 2-6 part of the acid α-glucosidase gene was isolated. Second, plasmid pαgluex2 was digested with SalI and NotI to obtain the fragment containing the 3' part of the acid α-glucosidase gene. Finally, the cosmid vector pWE15 was digested with NotI and dephosphorylated. These three fragments were ligated and packaged. The resulting cosmid is c8αgluex2.

Example 2

Transgenesis

The cDNA and genomic constructs were linearized with NotI and injected in the pronucleus of fertilized mouse oocytes which were then implanted in the uterus of pseudopregnant mouse foster mothers. The offspring were analyzed for the insertion of the human α-glucosidase cDNA or genomic DNA gene construct by Southern blotting of DNA isolated from clipped tails. Transgenic mice were selected and bred.

The genomic constructs linearized with NotI were also injected into the pronucleus of fertilized rabbit oocytes, which were implanted in the uterus of pseudopregnant rabbit foster mothers. The offspring were analyzed for the insertion of the alpha-glucosidase DNA by Southern blotting. Transgenic rabbits were selected and bred.

Example 3

Analysis of Acid α-glucosidase in the Milk of Transgenic Mice

Milk from transgenic mice and nontransgenic controls was analyzed by Western Blotting. The probe was mouse antibody specific for human acid α-glucosidase (i.e., does not bind to the mouse enzyme). Transgenes 1672 and 1673 showed expression of human acid α-glucosidase in milk (FIGS. **4**A and **4**B). Major and minor bands at 100-110 kD and 76 kD were observed as expected for α-glucosidase. In milk from non-transgenic mice, no bands were observed.

The activity of human acid α-glucosidase was measured with the artificial substrate 4-methylumbelliferyl-α-D-glucopyranoside in the milk of transgenic mouse lines (See Galiaard, Genetic Metabolic Disease, Early Diagnosis and Prenatal Analysis, Elsevier/North Holland, Amsterdam, pp. 809-827 (1980)). Mice containing the cDNA construct (FIG. **1**) varied from 0.2 to 2 .μmol/ml per hr. The mouse lines containing the genomic construct (FIG. **2**, panel A) expressed at levels from 10 to 610 .μmol/ml per hr. These figures are equivalent to a production of 1.3 to 11.3 mg/l (cDNA construct) and 0.05 to 3.3 g/l (genomic construct) based on an estimated specific activity of the recombinant enzyme of 180 .μmol/mg (Van der Ploeg et al., J. Neurol. 235:392-396 (1988)).

The recombinant acid α-glucosidase was isolated from the milk of transgenic mice, by sequential chromatography of milk on ConA-Sepharose™ and Sephadex™ G200. 7 ml milk was diluted to 10 ml with 3 ml Con A buffer consisting of 10 mM sodium phosphate, pH 6.6 and 100 mM NaCl. A 1:1 suspension of Con A sepharose in Con A buffer was then added and the milk was left overnight at 4.degree. C. with gentle shaking. The Con A sepharose beads were then collected by centrifugation and washed 5 times with Con A buffer, 3 times with Con A buffer containing 1 M NaCl instead of 100 mM, once with Con A buffer containing 0.5 M NaCl instead of 100 mM and then eluted batchwise with Con A buffer containing 0.5 M NaCl and 0.1 M methyl-α-D-mannopyranoside. The acid α-glucosidase activity in the eluted samples was measured using the artificial 4-methyl-umbelliferyl-α-D-glycopyranoside substrate (see above). Fractions containing acid α-glucosidase activity were pooled, concentrated and dialyzed against Sephadex buffer consisting of 20 mM Na acetate, pH 4.5 and 25 mM NaCl, and applied to a Sephadex™ 200 column. This column was run with the same buffer, and fractions were assayed for acid α-glucosidase activity and protein content. Fractions rich in acid α-glucosidase activity and practically free of other proteins were pooled and concentrated. The method as described is essentially the same as the one published by Reuser et al., Exp. Cell Res. 155:178-179 (1984). Several modifications of the method are possible regarding the exact composition and pH of the buffer systems and the choice of purification steps in number and in column material.

Acid α-glucosidase purified from the milk was then tested for phosphorylation by administrating the enzyme to cultured fibroblasts from patients with GSD II (deficient in endogenous acid α-glucosidase). In this test mannose 6-phosphate containing proteins are bound by mannose 6-phosphate receptors on the cell surface of fibroblasts and are subsequently internalized. The binding is inhibited by free mannose 6-phosphate (Reuser et al., Exp. Cell Res. 155:178-189 (1984)). In a typical test for the phosphorylation of acid α-glucosidase isolated from milk of transgenic mice, the acid α-glucosidase was added to 104-106 fibroblasts in 500 .μl culture medium (Ham F10, supplied with 10% fetal calf serum and 3 mM Pipes) in an amount sufficient to metabolize 1 μmole 4-methyl-umbelliferyl-α-D-glucopyranoside per hour for a time period of 20 hours. The experiment was performed with or without 5 mM mannose 6-phosphate as a competitor, essentially as described by Reuser et al., supra (1984). Under these conditions acid α-glucosidase of the patient fibroblasts was restored to the level measured in fibroblasts from healthy individuals. The restoration of the endogenous acid α-glucosidase activity by acid α-glucosidase isolated from mouse milk was as efficient as restoration by acid α-glucosidase purified from bovine testis, human urine and medium of transfected CHO cells. Restoration by α-glucosidase form milk was inhibited by 5 mM mannose 6-phosphate as observed for α-glucosidase from other sources. (Reuser et al., supra; Van der Ploeg et al., (1999), supra; Van der Ploeg et al., Ped. Res. 24:90-94 (198B).

As a further demonstration of the authenticity of α-glucosidase produced in the milk, the N-terminal amino acid sequence of the recombinant α-glucosidase produced in the milk of mice was shown to be the same as that of α-glucosidase precursor from human urine as published by Hoefsloot et al., EMBO J. 7:1697-1704 (1988) which starts with AHPGRP (SEQ ID NO:1).

Example 4

Animal Trial of Alpha-Glucosidase

Recently, a knock-out mouse model for Pompe's disease has become available (25) This model was generated by targeted disruption of the murine alpha-glucosidase gene. Glycogen-containing lysosomes are detected soon after birth in liver, heart and skeletal muscle. Overt clinical symptoms only become apparent at relatively late age (>9 months), but the heart is typically enlarged and the electrocardiogram is abnormal.

Experiments have been carried out using the knock-out (KO) mouse model in order to study the in vivo effect of AGLU purified from transgenic rabbit milk. The recombinant enzyme in these experiments was purified from milk of the transgenic rabbits essentially as described above for purification from transgenic mice.

1. Short Term Studies in KO Mouse Model

Single or multiple injections with a 6 day interval were administered to KO mice via the tail vein. Two days after the last enzyme administration the animals were killed, and the organs were perfused with phosphate buffered saline (PBS). Tissue homogenates were made for GLU enzyme activity assays and tissue glycogen content, and ultrathin sections of various organs were made to visualize accumulation (via electron microscopy) lysosomal glycogen content. Also the localization of internalized AGLU was determined using rabbit polyclonal antibodies against human placenta mature α-glucosidase.

The results showed that single doses of 0.7 and 1.7 mg AGLU (experiments C and A respectively) was taken up efficiently in vivo in various organs of groups of two knock-out mice when injected intravenously. Experiment A also showed that there were no differences in the uptake and distribution of AGLU purified from two independent rabbit milk sources.

Increases in AGLU activity were seen in the organs such as the liver, spleen, heart, and skeletal muscle, but not in the brain. Two days after a single injection of 1.7 mg AGLU to two KO animals, levels close to, or much higher than, the endogenous alpha-glucosidase activity levels observed in

organs of two PBS-injected normal control mice or two heterozygous KO mice were obtained (experiment A). Of the organs tested, the liver and spleen are, quantitatively, the main organs involved in uptake, but also the heart and pectoral and femoral muscles take up significant amounts of enzyme. The absence of a significant increase in brain tissue suggests that AGLU does not pass the blood-brain barrier. The results are summarized in Table 2.

Immunohistochemical staining of AGLU was evident in lysosomes of hepatocytes using a polyclonal rabbit antibody against human alpha-glucosidase. The presence of AGLU in heart and skeletal tissues is more difficult to visualize with this technique, apparently due to the lower uptake.

2. Long-Term Experiments with the KO Mouse Model

In longer term experiments, enzyme was injected in the tail vein of groups of two or three KO mice, once a week for

TABLE 2

Tissue Uptake of AGLU and Glycogen Content Following Short Term Treatment in KO Mouse Model

	Liver		Spleen		Heart		Pectoral Muscle		Femoral Muscle		Tongue		Brain	
Group	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc
Experiment A animals treated with single dose of 1.7 mg AGLU (from 2 sources)														
treated KO	674	—	—	—	263	—	—	—	24	—	—	—	0.8	—
mice source 1	410				17				3.1				0.4	
treated KO	454	—	—	—	76	—	—	—	12	—	—	—	0.8	—
mice source 2	604				48				10				0.4	
untreated KO mouse	3.1	—	—	—	0.2	—	—	—	0.2	—	—	—	0.2	—
untreated	58	—	—	—	23	—	—	—	11	—	—	—	57	—
normal mouse	37				17				8.2				57	
Experiment B animals treated with 4 doses of AGLU (1.0, 2.0, 1.0 and 1.4 mg.) 6 days apart														
treated KO	1132	70	—	—	24	1259	125	87	—	—	89	—	0.4	163
mice (13 weeks old)	944	13			10	1082	46	116			35		0.2	163
treated KO mice (34 weeks old)	3375	23	—	—	60	1971	49	90	—	—	207	—	0.7	374
untreated KO	2.0	406	—	—	0.2	3233	1.0	86	—	—	1.0	—	0.2	487
mice (13 and 34 weeks old)	2.0	147			0.3	1748	1.0	87			1.0		0.2	168
untreated normal mice (34 weeks old)	35	6	—	—	8.2	0	6.0	1.0	—	—	14	—	18	0
treated KO	582	—	462	—	46	—	—	—	5.1	—	—	—	0.4	—
mice	558		313		50				3.6				0.4	
untreated KO	1.1	—	0.8	—	0.3	—	—	—	0.2	—	—	—	0.2	—
mice	1.6		0.7		0.3				0.3				0.2	

Figures in the table refer to individual animals
Act: AGLU activity (nmoles 4MU per mg protein per hour)
GLC: Glycogen content (μg/mg protein)
n.d. not detected
— data unavailable

When two KO mice were injected 4 times every 6 days (experiment B), a marked decrease of total cellular glycogen was observed in both heart and liver. No effects were observed in skeletal muscle tissues with regard to total glycogen. However, in general the uptake of AGLU in these tissues was lower than in the other tissues tested.

Transmission electron microscopy of the 4 times injected KO mice indicated a marked decrease in lysosomal glycogen in both liver cells and heart muscle cells. The effects observed in heart tissue are localized since in some areas of the heart no decrease in lysosomal glycogen was observed after these short term administrations.

Western blot analysis using rabbit polyclonal antibodies against human placenta mature alpha-glucosidase indicated complete processing of the injected AGLU towards the mature enzyme in all organs tested strongly suggesting uptake in target tissues, and lysosomal localization and processing. No toxic effects were observed in any of the three experiments.

periods of up to 25 weeks. The initial dose was 2 mg (68 mg/kg) followed by 0.5 mg (17 mg/kg)/mouse for 12 weeks. In two groups of mice, this was followed by either 4 or 11 additional weeks of treatment of 2 mg/mouse. Injections started when the mice were 6-7 months of age. At this age, clear histopathology has developed in the KO model. Two days after the last enzyme administration the animals were killed, and the organs were perfused with phosphate buffered saline (PBS). Tissue homogenates were made for AGLU enzyme activity assays and tissue glycogen content, and sections of various organs were made to visualize (via light microscopy) lysosomal glycogen accumulation.

The results showed that mice treated 13 weeks with 0.5 mg/mouse (Group A, 3 animals/Group) had an increase of activity in the liver and spleen and decreased levels of glycogen in liver and perhaps in heart. One animal showed increased activity in muscles, although there was no significant decrease of glycogen in muscle.

Mice that were treated 14 weeks with 0.5 mg/mouse followed by 4 weeks with 2 mg/mouse (Group B, 3 animals/

Group) showed similar results to those treated for 13 weeks only, except that an increased activity was measured in the heart and skeletal muscles and decreases of glycogen levels were also seen in the spleen.

Mice that were treated 14 weeks with 0.5 mg/mouse followed by 11 weeks with 2 mg/mouse (Group C 2 animals/Group) showed similar results to the other two groups except that treated mice showed definite decreases in glycogen levels in liver, spleen, heart and skeletal muscle. No activity could be detected, even at the highest dose, in the brain.

Results of treated and untreated animals in each Group (Group means) are summarized in Table 3.

TABLE 3

Tissue Uptake of AGLU and Glycogen Content Following Long Term Treatment in KO Mouse Model

	Liver		Spleen		Heart		Pectoral Muscle		Quadriceps Muscle		Gastroenemius Muscle		Brain	
Group	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc	Act	Glc
Group A animals treated with 0.5 mg/mouse/week for 13 weeks														
treated	713	2	463	n.d.	3	86	9	81	6	40	14	66	—	—
untreated	2	24	1	n.d.	1	111	1	66	1		1	61	—	—
Group B animals treated with 0.5 mg/mouse/week for 14 weeks, followed by 2 mg/mouse/week for 4 weeks														
treated	2705	1	1628	0	59	288	49	120	30	128	44	132	—	—
untreated	3	11	31	6	1	472	1	113	1	162	1	142	—	—
Group C animals treated with 0.5 mg/mouse/week for 14 weeks, followed by 2 mg/mouse/week for 11 weeks														
treated	1762	1	1073	2	66	211	99	113	37	18	109	32	1	32
untreated	2	45	1	21	1	729	1	291	0	104	0	224	0	44

Figures in the table refer to the mean of 3animals (Groups A and B) or the mean of 2 animals (Group C)
Act: AGLU activity (nmoles 4MU per mg protein per hour)
GLC: Glycogen content (μg/mg protein)
n.d. not detected
— data unavailable

In addition, a very convincing improvement in the histopathological condition was observed in Group C mice (treated for the first 14 weeks at 0.5 mg/mouse, followed by 11 weeks at 2 mg/mouse). Clear reversal of pathology was demonstrated in various tissues, such as heart and pectoralis muscle.

It has been reported that Pompe's disease does not occur when the residual lysosomal α-glucosidase activity is >20% of average control value (14). The data obtained with the KO mouse model indicates that these levels are very well achievable using recombinant precursor enzyme.

Example 5

Human Clinical Trial

A single phase I study (AGLUI 101-01) has been conducted in 15 healthy male volunteers. Doses of AGLU ranged from 25 to 800 mg, administered by intravenous infusion to healthy male adult volunteers. Subjects with a history of allergies and hypersensitivities were excluded from the study. The subjects were randomized into dose groups of 5, and each dose Group received AGLU (4 subjects) or placebo (1 subject) at each dose level. All subjects received two doses of study drug, which were administered two weeks apart. The dosing regimen was as follows:

A
25 mg: Group 1, treatment period 1
B
50 mg: Group 1, treatment period 2
C
100 mg: Group 2, treatment period 1
D
200 mg: Group 3, treatment period b **1**
E
400 mg: Group 2, treatment period 2
F
800 mg: Group 3, treatment period 2
P
placebo (1 subject per Group and treatment period)

Subjects were administered AGLU or placebo as an infusion on Day 1 of each treatment period. The infusions were administered over a 30 minute period and subjects were kept in a semi-recumbent position for at least 2 hours after cessation of infusion.

Adverse events were recorded just before the start of the infusion, at 30 minutes (end of infusion) and at 3, 12, 24, 36 and 48 hours thereafter as well as on Days 5 and 8 (first period) and days 5, 8 and 15 (second period). Vital signs, ECG and physical examinations were also monitored regularly throughout the treatment period.

Blood samples were taken for a standard range of clinical laboratory tests and pharmacokinetics analysis. The subject's urine was collected and a standard range of laboratory analyses (including determination of AGLU) were performed.

(a) Laboratory Safety and Adverse Events

There were no clinically significant changes in laboratory parameters, clinical signs and ECG measurements in any subjects at any dose Group. The results of adverse event monitoring in all subjects at all doses are summarized in Table 4.

TABLE 4

Adverse Event Reports

Dose (mg)	Adverse Events
25	The reported events were muscle weakness, abnormal vision and fatigue. All events were mild and were deemed unrelated to the test article by the investigator

TABLE 4-continued

Adverse Event Reports	
Dose (mg)	Adverse Events
50	The reported events were headache, rhinitis, nose bleed and paresthesia. All events were mild and were deemed unrelated or remotely related to the test article by the investigator, except the paresthesia which was classed as moderate and was deemed possibly related to the test article.
100	The reported events were rhinitis, headache, fatigue, hematoma and injection site reaction. All events were classed as mild. The cases of hematoma, injection site reaction and intermittent headache were deemed possibly or probably related to the test article by the investigator. The other events were deemed to be unrelated.
200	The reported events were nausea, headache, dizziness, fatigue, rhinitis, photophobia, vision abnormalities and euphoria. All events were classed as mild or moderate in intensity. Seven events (including cases of dizziness, nausea and abnormal vision) were deemed to be possibly or probably related to the test article.
400	The reported events were fatigue and paresthesia. The report of fatigue was considered unrelated to the test article, and the paresthesia was deemed possibly related.
800	The reported events were nausea, drowsiness, dizziness, increased sweating, asthenia, shivering and intermittent headache. All events were classed as mild or moderate in intensity. Eight events (including cases of drowsiness, nausea and asthenia) were deemed to be possibly related to the test article.

A trial of the safety and efficacy of recombinant acid α-glucosidase as enzyme replacement therapy on infantile and juvenile patients with glycogen storage disease Type II is conducted. Four infantile patients and three juvenile patients are recruited. Infantiles are administered a starting dose of 15-20 mg/kg titrated to 40 mg/kg and juveniles are administered 10 mg/kg. Patients are treated for 24 weeks.

Patients are evaluated by the following parameters.

Standard adverse event reporting including suspected adverse events

Laboratory parameters including hematology, clinical chemistry and antibody detection.

α-glucosidase activity in muscle

Muscle histopathology

12-lead ECG

Clinical condition including neurological examination

Non-parametric PK parameters

Life saving interventions

Infantile patients are evaluated for the following additional parameters.

Left posterior ventricular wall thickness and left ventricular mass index

Neuromotor development

Survival

Glycogen content in muscle

Juvenile patients are evaluated for the following additional parameters.

Pulmonary function

Muscle strength/timed tests and muscle function

PEDI/Rotterdam 9-item scale

The same patients are then subject to additional dosages of alpha glucosidase with infantiles receiving 15, 20, 30 or 40 mg/kg and juveniles: 10 mg/kg for an additional period of 24 weeks and evaluated by the parameters indicated above.

A further phase II clinical trial is performed on eight patients aged <6 months of age within 2 months after diagnosis at a dosage of 40 mg/kg. Patients are treated for 24 weeks and evaluated by the following criteria:

Safety parameters

Laboratory safety data

Adverse event recording

Primary efficacy parameter: survival without life-saving interventions (i.e. mechanical ventilation >24 hr) 6 months past diagnosis in combination with normal or mildly delayed motor function (BSID II).

Secondary efficacy: Changes in neuromotor development, changes in left posterior ventricular wall thickness and left ventricular mass index; Changes in skeletal muscle acid α-glucosidase activity and glycogen content.

Efficacy can be show by a 50% survival at 6 months post-diagnosis without life saving interventions in the α-glucosidase group compared to 10% survival in the historical control group in combination with a BSID II classified as normal or mildly delayed.

A further clinical trial is performed on juvenile patients. The patients are aged >1 year and <35 years of age with juvenile onset of GSD type IIb The patients are administered 10 mg/kg or 20 mg/kg for a period of twenty-four weeks treatment. Treatment is monitored by the following parameters.

Safety parameters	Laboratory safety data Adverse event recording
Primary efficacy	Pulmonary function parameters (e.g. FVC, time on ventilator) Muscle strength
Secondary efficacy	Life-saving interventions parameters: Quality of life Skeletal muscle acid α-glucosidase activity
Quantitative objective	20% relative improvement in primary efficacy parameters over baseline

All quantitative measurements relating to efficacy are preferably statistically significant relative to contemporaneous or historical controls, preferably at $p<0.05$.

Example 6

Pharmaceutical Formulations

Alpha-glucosidase is formulated as follows: 5 mg/ml α-Glu, 15 mM sodium phosphate, pH 6.5, 2% (w/w) mannitol, and 0.5% (w/w) sucrose. The above formulation is filled to a final volume of 10.5 ml into a 20 cc tubing vial and lyophilized. For testing, release and clinical use, each vial is reconstituted with 10.3 ml* of sterile saline (0.9%) for injection (USP or equivalent.) to yield 10.5 ml of a 5 mg/ml α-Glu solution that may be directly administered or subsequently diluted with sterile saline to a patient specific target dose concentration. The 10.5 ml fill (52.5 mg alpha glucosidase total in vial) includes the USP recommended overage, that allows extraction and delivery (or transfer) of 10 mls (50 mg). The mannitol serves as a suitable bulling agent shortening the lyophilization cycle (relative to sucrose alone). The sucrose serves as a cryo/lyoprotectant resulting in no significant increase in aggregation following reconstitution. Reconstitution of the mannitol (only) formulations had repeatedly resulted in a slight increase in aggregation. Following lyophilization, the cake quality was acceptable and subsequent reconstitution times were significantly reduced Saline is preferred to HSA/dextrose for infusion solution. When saline is used in combination with lyophilization in 2% mannitol/0.5% sucrose the solution has acceptable tonicity for intravenous administration. The lyophilized vials containing the 2% mannitol/0.5% sucrose formulation were reconstituted with 0.9% sterile saline (for injection) to yield 5 mg/ml α-Glu.

Example 7

Infusion Schedule

The solution is administered via the indwelling intravenous cannula. Patients are monitored closely during the infusion period and appropriate clinical intervention are taken in the event of an adverse event or suspected adverse event. A window of 48 hours is allowed for each infusion. An infusion schedule in which the rate of infusion increases with time reduces or eliminates adverse events.

Infusions for infantiles can be administered according to the following schedule:

5 cc/hr for 60 minutes

10 cc/hr for 60 minutes

gtoreq.40 cc/hr for 30 minutes

gtoreq.80 cc/hr for the remainder of the infusion

Infusions for juveniles can be administered according to the following schedule:

0.5 cc/kg/hr for 60 minutes

1 cc/kg/hr for 60 minutes

5 cc/kg/hr for 30 minutes

7.5 cc/kg hr for the remainder of the infusion

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

SEQUENCE LISTING

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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: N-terminal of recombinant alpha-glucosidase

<400> SEQUENCE: 1

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1 5

<210> SEQ ID NO 2
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<223> OTHER INFORMATION: Figure 1: flanking sequence

<400> SEQUENCE: 2

ctcgagtatc gattgaattc atctgtcgac gctacc 36

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<220> FEATURE:
<223> OTHER INFORMATION: Figure 1: flanking sequence

<400> SEQUENCE: 3

gcatgcctcg acggtacc 18

What is claimed is:

1. A method of treating a human patient with Pompe's disease, comprising administering intravenously to the patient a therapeutically effective amount of human acid alpha glucosidase, whereby the concentration of accumulated glycogen in the patient is reduced and/or further accumulation of glycogen is arrested.

2. The method of claim **1**, wherein the alpha-glucosidase is produced in milk of a transgenic mammal.

3. The method of claim **1**, wherein the alpha-glucosidase is predominantly in a 110 kD form.

4. The method of claim **1**, wherein the alpha-glucosidase is administered weekly.

5. The method of claim **4**, wherein the therapeutically effective amount of human acid alpha-glucosidase is at least 10 mg/kg body weight of the patient.

6. A method of treating a human patient with Pompe's disease, comprising intravenously administering biweekly to the patient a therapeutically effective amount of human acid alpha glucosidase, whereby hypertrophic cardiomyopathy in the patient is reduced and/or arrested.

* * * * *

STATUTORY AND REGULATORY ADDENDUM

STATUTORY AND REGULATORY ADDENDUM

Selected Excerpts From U.S. Code, Title 35

35 U.S.C. § 103. Conditions for patentability; non-obvious subject matter.

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

35 U.S.C. § 311. Inter partes review.

(a) In general.—Subject to the provisions of this chapter, a person who is not the owner of a patent may file with the Office a petition to institute an inter partes review of the patent. The Director shall establish, by regulation, fees to be paid by the person requesting the review, in such amounts as the Director determines to be reasonable, considering the aggregate costs of the review.

(b) Scope.—A petitioner in an inter partes review may request to cancel as unpatentable 1 or more claims of a patent only on a ground that could be raised under section 102 or 103 and only on the basis of prior art consisting of patents or printed publications.

(c) Filing deadline.—A petition for inter partes review shall be filed after the later of either—

(1) the date that is 9 months after the grant of a patent; or

(2) if a post-grant review is instituted under chapter 32, the date of the termination of such post-grant review.

35 U.S.C. § 312. Petitions.

(a) Requirements of petition.—A petition filed under section 311 may be considered only if—

(1) the petition is accompanied by payment of the fee established by the Director under section 311;

(2) the petition identifies all real parties in interest;

(3) the petition identifies, in writing and with particularity, each claim challenged, the grounds on which the challenge to each claim is based, and the evidence that supports the grounds for the challenge to each claim, including—

(A) copies of patents and printed publications that the petitioner relies upon in support of the petition; and

(B) affidavits or declarations of supporting evidence and opinions, if the petitioner relies on expert opinions;

(4) the petition provides such other information as the Director may require by regulation; and

(5) the petitioner provides copies of any of the documents required under paragraphs (2), (3), and (4) to the patent owner or, if applicable, the designated representative of the patent owner.

(b) Public availability.—As soon as practicable after the receipt of a petition under section 311, the Director shall make the petition available to the public.

35 U.S.C. § 313. Preliminary response to petition.

If an inter partes review petition is filed under section 311, the patent owner shall have the right to file a preliminary response to the petition, within a time period set by the Director, that sets forth reasons why no inter partes review should be instituted based upon the failure of the petition to meet any requirement of this chapter.

35 U.S.C. § 314. Institution of inter partes review.

(a) Threshold.—The Director may not authorize an inter partes review to be instituted unless the Director determines that the information presented in the petition filed under section 311 and any response filed under section 313 shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition.

(b) Timing.—The Director shall determine whether to institute an inter partes review under this chapter pursuant to a petition filed under section 311 within 3 months after—

(1) receiving a preliminary response to the petition under section 313; or

(2) if no such preliminary response is filed, the last date on which such response may be filed.

(c) Notice.—The Director shall notify the petitioner and patent owner, in writing, of the Director's determination under subsection (a), and shall make such notice available to the public as soon as is practicable. Such notice shall include the date on which the review shall commence.

(d) No appeal.—The determination by the Director whether to institute an inter partes review under this section shall be final and nonappealable.

35 U.S.C. § 315. Relation to other proceedings or actions.

(a) Infringer's civil action.—

(1) Inter partes review barred by civil action.—An inter partes review may not be instituted if, before the date on which the petition for such a review is filed, the petitioner or real party in interest filed a civil action challenging the validity of a claim of the patent.

(2) Stay of civil action.—If the petitioner or real party in interest files a civil action challenging the validity of a claim of the patent on or after the date on which the petitioner files a petition for inter partes review of the patent, that civil action shall be automatically stayed until either—

(A) the patent owner moves the court to lift the stay;

(B) the patent owner files a civil action or counterclaim alleging that the petitioner or real party in interest has infringed the patent; or

(C) the petitioner or real party in interest moves the court to dismiss the civil action.

(3) Treatment of counterclaim.—A counterclaim challenging the validity of a claim of a patent does not constitute a civil action challenging the validity of a claim of a patent for purposes of this subsection.

(b) Patent owner's action.—An inter partes review may not be instituted if the petition requesting the proceeding is filed more than 1 year after the date on which the petitioner, real party in interest, or privy of the petitioner is served with a complaint alleging infringement of the patent. The time limitation set forth in the preceding sentence shall not apply to a request for joinder under subsection (c).

(c) Joinder.—If the Director institutes an inter partes review, the Director, in his or her discretion, may join as a party to that inter partes review any person who

properly files a petition under section 311 that the Director, after receiving a preliminary response under section 313 or the expiration of the time for filing such a response, determines warrants the institution of an inter partes review under section 314.

(d) Multiple proceedings.—Notwithstanding sections 135(a), 251, and 252, and chapter 30, during the pendency of an inter partes review, if another proceeding or matter involving the patent is before the Office, the Director may determine the manner in which the inter partes review or other proceeding or matter may proceed, including providing for stay, transfer, consolidation, or termination of any such matter or proceeding.

(e) Estoppel.—

(1) Proceedings before the Office.—The petitioner in an inter partes review of a claim in a patent under this chapter that results in a final written decision under section 318(a), or the real party in interest or privy of the petitioner, may not request or maintain a proceeding before the Office with respect to that claim on any ground that the petitioner raised or reasonably could have raised during that inter partes review.

(2) Civil actions and other proceedings.—The petitioner in an inter partes review of a claim in a patent under this chapter that results in a final written decision under section 318(a), or the real party in interest or privy of the petitioner, may not assert either in a civil action arising in whole or in part under section 1338 of title 28 or in a proceeding before the International Trade Commission under section 337 of the Tariff Act of 1930 that the claim is invalid on any ground that the petitioner raised or reasonably could have raised during that inter partes review.

35 U.S.C. § 316. Conduct of inter partes review.

(a) Regulations.—The Director shall prescribe regulations—

(1) providing that the file of any proceeding under this chapter shall be made available to the public, except that any petition or document filed with the intent that it be sealed shall, if accompanied by a motion to seal, be treated as sealed pending the outcome of the ruling on the motion;

(2) setting forth the standards for the showing of sufficient grounds to institute a review under section 314(a);

(3) establishing procedures for the submission of supplemental information after the petition is filed;

(4) establishing and governing inter partes review under this chapter and the relationship of such review to other proceedings under this title;

(5) setting forth standards and procedures for discovery of relevant evidence, including that such discovery shall be limited to—

(A) the deposition of witnesses submitting affidavits or declarations; and

(B) what is otherwise necessary in the interest of justice;

(6) prescribing sanctions for abuse of discovery, abuse of process, or any other improper use of the proceeding, such as to harass or to cause unnecessary delay or an unnecessary increase in the cost of the proceeding;

(7) providing for protective orders governing the exchange and submission of confidential information;

(8) providing for the filing by the patent owner of a response to the petition under section 313 after an inter partes review has been instituted, and requiring that the patent owner file with such response, through affidavits or declarations, any additional factual evidence and expert opinions on which the patent owner relies in support of the response;

(9) setting forth standards and procedures for allowing the patent owner to move to amend the patent under subsection (d) to cancel a challenged claim or propose a reasonable number of substitute claims, and ensuring that any information submitted by the patent owner in support of any amendment entered under subsection (d) is made available to the public as part of the prosecution history of the patent;

(10) providing either party with the right to an oral hearing as part of the proceeding;

(11) requiring that the final determination in an inter partes review be issued not later than 1 year after the date on which the Director notices the institution of a review under this chapter, except that the Director may, for good cause shown, extend the 1-year period by not more than 6 months, and may adjust the time periods in this paragraph in the case of joinder under section 315(c);

(12) setting a time period for requesting joinder under section 315(c); and

(13) providing the petitioner with at least 1 opportunity to file written comments within a time period established by the Director.

(b) Considerations.—In prescribing regulations under this section, the Director shall consider the effect of any such regulation on the economy, the integrity of

the patent system, the efficient administration of the Office, and the ability of the Office to timely complete proceedings instituted under this chapter.

(c) Patent Trial and Appeal Board.—The Patent Trial and Appeal Board shall, in accordance with section 6, conduct each inter partes review instituted under this chapter.

(d) Amendment of the patent.—

(1) In general.—During an inter partes review instituted under this chapter, the patent owner may file 1 motion to amend the patent in 1 or more of the following ways:

(A) Cancel any challenged patent claim.

(B) For each challenged claim, propose a reasonable number of substitute claims.

(2) Additional motions.—Additional motions to amend may be permitted upon the joint request of the petitioner and the patent owner to materially advance the settlement of a proceeding under section 317, or as permitted by regulations prescribed by the Director.

(3) Scope of claims.—An amendment under this subsection may not enlarge the scope of the claims of the patent or introduce new matter.

(e) Evidentiary standards.—In an inter partes review instituted under this chapter, the petitioner shall have the burden of proving a proposition of unpatentability by a preponderance of the evidence.

35 U.S.C. § 318. Decision of the Board.

(a) Final written decision.—If an inter partes review is instituted and not dismissed under this chapter, the Patent Trial and Appeal Board shall issue a final written decision with respect to the patentability of any patent claim challenged by the petitioner and any new claim added under section 316(d).

(b) Certificate.—If the Patent Trial and Appeal Board issues a final written decision under subsection (a) and the time for appeal has expired or any appeal has terminated, the Director shall issue and publish a certificate canceling any claim of the patent finally determined to be unpatentable, confirming any claim of the patent determined to be patentable, and incorporating in the patent by operation of the certificate any new or amended claim determined to be patentable.

(c) Intervening rights.—Any proposed amended or new claim determined to be patentable and incorporated into a patent following an inter partes review under this chapter shall have the same effect as that specified in section 252 for reissued patents on the right of any person who made, purchased, or used within the United States, or imported into the United States, anything patented by such proposed amended or new claim, or who made substantial preparation therefor, before the issuance of a certificate under subsection (b).

(d) Data on length of review.—The Office shall make available to the public data describing the length of time between the institution of, and the issuance of a final written decision under subsection (a) for, each inter partes review.

35 U.S.C. § 319. Appeal.

A party dissatisfied with the final written decision of the Patent Trial and Appeal Board under section 318(a) may appeal the decision pursuant to sections 141 through 144. Any party to the inter partes review shall have the right to be a party to the appeal.

Selected Excerpts From The Administrative Procedure Act

5 U.S.C. § 554. Adjudications.

(a) This section applies, according to the provisions thereof, in every case of adjudication required by statute to be determined on the record after opportunity for an agency hearing, except to the extent that there is involved—

(1) a matter subject to a subsequent trial of the law and the facts de novo in a court;

(2) the selection or tenure of an employee, except a1 administrative law judge appointed under section 3105 of this title;

(3) proceedings in which decisions rest solely on inspections, tests, or elections;

(4) the conduct of military or foreign affairs functions;

(5) cases in which an agency is acting as an agent for a court; or

(6) the certification of worker representatives.

(b) Persons entitled to notice of an agency hearing shall be timely informed of—

(1) the time, place, and nature of the hearing;

(2) the legal authority and jurisdiction under which the hearing is to be held; and

(3) the matters of fact and law asserted.

When private persons are the moving parties, other parties to the proceeding shall give prompt notice of issues controverted in fact or law; and in other instances agencies may by rule require responsive pleading. In fixing the time and place for hearings, due regard shall be had for the convenience and necessity of the parties or their representatives.

(c) The agency shall give all interested parties opportunity for—

(1) the submission and consideration of facts, arguments, offers of settlement, or proposals of adjustment when time, the nature of the proceeding, and the public interest permit; and

(2) to the extent that the parties are unable so to determine a controversy by consent, hearing and decision on notice and in accordance with sections 556 and 557 of this title.

* * *

5 U.S.C. § 557. Initial decisions; conclusiveness; review by agency; submissions by parties; contents of decisions; record.

(a) This section applies, according to the provisions thereof, when a hearing is required to be conducted in accordance with section 556 of this title.

* * *

(c) Before a recommended, initial, or tentative decision, or a decision on agency review of the decision of subordinate employees, the parties are entitled to a reasonable opportunity to submit for the consideration of the employees participating in the decisions—

(1) proposed findings and conclusions; or

(2) exceptions to the decisions or recommended decisions of subordinate employees or to tentative agency decisions; and

(3) supporting reasons for the exceptions or proposed findings or conclusions.

The record shall show the ruling on each finding, conclusion, or exception presented. All decisions, including initial, recommended, and tentative decisions, are a part of the record and shall include a statement of—

(A) findings and conclusions, and the reasons or basis therefor, on all the material issues of fact, law, or discretion presented on the record; and

(B) the appropriate rule, order, sanction, relief, or denial thereof.

*** * ***

5 U.S.C. § 706. Scope of Review.

To the extent necessary to decision and when presented, the reviewing court shall decide all relevant questions of law, interpret constitutional and statutory provisions, and determine the meaning or applicability of the terms of an agency action. The reviewing court shall—

(1) compel agency action unlawfully withheld or unreasonably delayed; and

(2) hold unlawful and set aside agency action, findings, and conclusions found to be—

(A) arbitrary, capricious, an abuse of discretion, or otherwise not in accordance with law;

(B) contrary to constitutional right, power, privilege, or immunity;

(C) in excess of statutory jurisdiction, authority, or limitations, or short of statutory right;

(D) without observance of procedure required by law;

(E) unsupported by substantial evidence in a case subject to sections 556 and 557 of this title or otherwise reviewed on the record of an agency hearing provided by statute; or

(F) unwarranted by the facts to the extent that the facts are subject to trial de novo by the reviewing court.

In making the foregoing determinations, the court shall review the whole record or those parts of it cited by a party, and due account shall be taken of the rule of prejudicial error.

Selected Regulatory Excerpts

37 C.F.R. § 42.1. Policy.

(a) Scope. Part 42 governs proceedings before the Patent Trial and Appeal Board. Sections 1.4, 1.7, 1.14, 1.16, 1.22, 1.23, 1.25, 1.26, 1.32, 1.34, and 1.36 of this chapter also apply to proceedings before the Board, as do other sections of part 1 of this chapter that are incorporated by reference into this part.

(b) Construction. This part shall be construed to secure the just, speedy, and inexpensive resolution of every proceeding.

* * *

37 C.F.R. § 42.2. Definitions.

The following definitions apply to this part:

* * *

Trial means a contested case instituted by the Board based upon a petition. A trial begins with a written decision notifying the petitioner and patent owner of the institution of the trial. The term trial specifically includes * * * an inter partes review under Chapter 31 of title 35, United States Code * * *.

37 C.F.R. § 42.4. Notice of Trial.

(a) Institution of trial. The Board institutes the trial on behalf of the Director.

(b) Notice of a trial will be sent to every party to the proceeding. The entry of the notice institutes the trial.

* * *

37 C.F.R. § 42.71. Decision on petitions or motions.

* * *

(d) Rehearing. A party dissatisfied with a decision may file a single request for rehearing without prior authorization from the Board. The burden of showing a decision should be modified lies with the party challenging the decision. The request must specifically identify all matters the party believes the Board misapprehended or overlooked, and the place where each matter was previously

addressed in a motion, an opposition, or a reply. A request for rehearing does not toll times for taking action. Any request must be filed:

(1) Within 14 days of the entry of a non-final decision or a decision to institute a trial as to at least one ground of unpatentability asserted in the petition; or

(2) Within 30 days of the entry of a final decision or a decision not to institute a trial.

37 C.F.R. § 42.120. Patent owner response.

(a) Scope. A patent owner may file a response to the petition addressing any ground for unpatentability not already denied. A patent owner response is filed as an opposition and is subject to the page limits provided in § 42.24.

* * *

CERTIFICATE OF COMPLIANCE

1. This brief complies with the type-volume limitations of Fed. R. App. P. 32(a)(7)(B) because this brief contains 13,795 words, excluding the parts of the brief exempted by Fed. R. App. P. 32(a)(7)(B)(iii).

2. This brief complies with the typeface requirements of Fed. R. App. P. 32(a)(5) and the type style requirements of Fed. R. App. P. 32(a)(6) because this brief has been prepared in a proportionally spaced typeface using Microsoft Word 2010 in Times New Roman 14-point font.

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Dated: August 17, 2015

CERTIFICATE OF FILING AND SERVICE

United States Court of Appeals for the Federal Circuit

Genzyme Therapeutic v. Biomarin Pharmaceutical, Inc., 2015-1720,-1721

CERTIFICATE OF SERVICE

I, Maryna Sapyelkina, being duly sworn according to law and being over the age of 18, upon my oath depose and say that:

Counsel Press was retained by O'Melveny & Myers LLP, Attorneys for Appellant to print this document. I am an employee of Counsel Press.

On the **17th Day of August, 2015**, counsel for Appellant has authorized me to electronically file the within **Brief for Appellant** with the Clerk of the Court using the CM/ECF System, which will serve via e-mail notice of such filing to any of the following counsel registered as CM/ECF users:

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Upon acceptance by the Court of the e-filed document, six paper copies of the brief will be filed with the Court, via Federal Express, within the time provided in the Court's rules.

August 17, 2015

/s/ Maryna Sapyelkina
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Counsel Press